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RESPIRATORY AND CIRCULATORY RESPONSES TO ACUTE CARBON MONOXIDE POISONING

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Asmussen and Chiodi (1) have reported that CO saturations ranging from 23 to 33 per cent have but slight effects on the circulatory and respiratory systems of man. The possibility that higher saturations with CO would lead to responses differing in type or degree led us to extend these studies. Observations have been made on pulmonary ventilation, cardiac output and plasma pH in man and dog. The effect of CO on the respiratory response to high CO_2 tension has been studied in man.

Haldane (7) was one of the first to study by self-experiment the effects of CO poisoning. His report, that a hyperventilation began at about 35 per cent saturation, is not in harmony with the clinical observations of Sayers et al. (13) and of Hayhurst (8) that CO poisoning does not influence respiration. However, Haggard and Henderson (6) found that in dogs 50 per cent or more saturated with CO there was sufficient hyperventilation to induce an alkalosis and a decreased blood alkali. Sectioning of the vagi prevented both these responses. Theil (16) also noted a hyperventilation in dogs but his data showed large and unexplained variations. The arterial CO_2 and pH both decreased, indicating a state of partially compensated acidosis. While Kamei (10) confirmed the occurrence of acidosis in rabbits and dogs, hyperventilation was not a constant feature, being present only when the concentration of CO in the inspired air was high.

The influence of CO on the circulatory system has received slight attention. Miura (11) found that in rabbits the cardiac output increased and the arterio-venous O_2 differences decreased with increased CO saturation of blood. In man, Asmussen and Chiodi (1) found only a slight increase of the cardiac output with CO poisoning ranging up to 33 per cent.

METHOD. The procedure followed was similar to that previously de-

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scribed (1) except that instead of breathing a CO-O_2 mixture in a closed circuit of small volume, a mixture of 0.15 to 0.35 per cent CO in air was inspired from a 600-liter Tissot. Since poisoning by this method was more gradual, we were able to obtain successive observations on ventilation, cardiac output and CO of venous blood. The details are as follows:

The subject, in the basal state, lay on the bed for approximately one hour before determinations were begun. Basal O_2 consumption, ventilation, pulse rate and blood pressure were recorded and arterial blood for pH was obtained. A basal cardiac output was then determined by Grollman's method.

The subject then began inspiring the CO-air mixture, or air alone, from the large gasometer, it being necessary to interrupt this only while making the observations on cardiac output. The total time during which the mixture was inhaled was never less than 70 minutes. The subject remained connected to the Tissot while the foregoing observations were repeated from one to three times during this period.

The procedure for determining the effect of CO on the respiratory response to CO_2 was as follows:

Pulmonary ventilation was measured breathing air and air-CO_2 mixtures. The subject was then given CO from a closed circuit filled with a CO-O_2 mixture. After 10 minutes ventilation was again measured using air and then the air-CO_2 mixture. The usual observations were made and samples of blood were taken for CO determination immediately before and after the final period of breathing the CO_2 mixture.

Experiments were also performed on dogs trained for metabolic observations. In the morning, after a fast of 16 hours, the unanesthetized dog was placed on a table and fitted with a leather mask over his muzzle. Lanolin and a rubber bandage made the mask airtight. The flow of inspired air from outdoors and of expired air into a gasometer was regulated by means of light rubber valves. Heart rates were recorded continuously by a Guillemin cardiotachometer.[2] The cardiac output was determined utilizing the Fick principle. Mixed venous blood was obtained from the right heart and arterial from the femoral artery. The procedure followed before and during the CO poisoning was similar to that used in the human subjects.

The methods of analysis employed have been previously described (1). The details of the determination of pH are to be found in the paper by Dill et al. (5), with the difference that the corrections for unsaturation were made on the basis of available hemoglobin capacity rather than on total hemoglobin capacity. The total hemoglobin was determined by saturating the blood with CO and analyzing it in the Van Slyke apparatus. Total ventilation was reduced to 0°C., 760 mm. Hg and dryness for calculating

[2] Manufactured by Victor Guillemin, Jr., 68 Jason St., Arlington, Mass.

the metabolism, and to 37°C., prevailing barometric pressure and complete saturation for estimating the minute ventilation.

RESULTS ON DOGS. The data on two Irish terrier bitches each weighing approximately 9.5 kgm. are shown in table 1. Lady had the same lung ventilation during CO poisoning as in the averaged control experiments, even though the saturation exceeded 50 per cent. In her the plasma pH showed a slight acid shift. In Dee the pH change was more pronounced. However, the lowest pH, 7.13, was found following a period of vomiting induced by the poisoning.

TABLE 1

Respiratory and circulatory effects of CO poisoning in dogs

DOG	EXPERIMENT NO.	VENTILATION	O_2 USED	A-V O_2 DIFF.	CARDIAC OUTPUT	PULSE RATE	pH_s	BLOOD LACTATE	TOTAL Hb CAPACITY	HbCO	REMARKS
		l. per min.	*cc. per min.*	*cc.*	*l. per min.*			*mgm. %*	*vol. %*	*%*	
Dee ♀	1–7		74	46	1.6	90	7.33		19.6	0	Average of 7
9.5 kgm.	8		61	37	1.6	145	7.28	10	21.4	20	experiments
	9		67	37	1.8				21.7	34	
	10		67	35	1.9	130			19.6	35	
	11		62	32	1.9	136			17.8	38	
	12		76	29	2.6	179	7.25		20.6	41	
	13		68	22	3.0				20.5	43	
	14		82	20	4.1	204		50	21.9	50	
	15		94	35	2.7	210	7.13	21	22.2	53	Vomited
Lady ♀	1–4	1.2	68	45	1.5	93	7.32	17	20.5	0	Average of 4
9.7 kgm.	5	1.4	66	45	1.4	106	7.30		22.3	25	experiments
	6	1.2	62	40	1.5	130	7.29	7	23.2	37	
	7	1.6	74	30	2.4	150	7.33	15	21.1	48	
	8	1.3	66	26	2.4	148	7.30	10	23.0	52	

There was a decrease in the average arterio-venous O_2 differences with higher CO. Although the cardiac output seemed to increase progressively, there was a rather abrupt rise when the hemoglobin saturation with CO exceeded 35 to 40 per cent. In three experiments on Dee with CO saturations of 35 to 38 per cent, the cardiac output ranged from 1.8 to 1.9 liters, but jumped to 2.6 liters when the HbCO was 41 per cent.

The blood lactates were within the normal range whenever determined, with only one exception. The heart rate tended to increase with a greater saturation of hemoglobin with CO.

RESULTS ON MEN. The four subjects were F. C., 28 years, 173 cm., 88 kgm.; S. H., 29 years, 162 cm., 74 kgm.; H. C., 33 years, 170 cm., 77 kgm.;

B. C., colored, 21 years, 170 cm., 64.5 kgm. The first three were subjects for one set of experiments, and the third and fourth for the other. When subjected to CO, the ventilation of H. C. decreased slightly (see tables 2 and 3). In F. C. no consistent differences were observed, and S. H. showed no respiratory response. The alkali reserve (T_{40}), measured on

TABLE 2

Arterial blood during CO poisoning in man

SUBJECT	EXPERIMENT NO.	VENTILATION	T_{40}*	pCO_2	$(BHCO_3)_a$	pH_a	TOTAL Hb CAPACITY	HbCO	O_2 ART. SATURATION	REMARKS
		l. per min.	*vol. %*	*mm. Hg*	*vol. %*		*vol. %*	*vol. %*	*%*	
H. C.	1		46.0	43.7	51.6	7.33	18.9	0	96.2	
			45.8	47.5	51.4	7.29	19.1	6.3	97.3	Recovery†
	2	7.2	47.5	41.9	54.1	7.37	19.3	0	97.7	
		6.9	47.8	40.0	55.2	7.39	20.6	7.2	90.7	Recovery
	3	7.0	46.3	46.3	52.4	7.31	19.4	0	97.1	
		6.5	46.7	45.1	53.2	7.33	19.9	4.6	98.3	0.15% CO in air‡
	4	6.9	47.6	42.8	54.2	7.36	19.6	0	96.6	
		6.7	47.4	45.6	54.1	7.33	20.0	9.8	97.4	0.3% CO in air
	5	6.6	48.1	39.3	55.1	7.40	19.1	0	95.7	
		6.9	47.4	43.7	54.0	7.35	19.8	9.2	95.2	0.3% CO in air
F. C.	1	8.2	47.2	43.8	53.8	7.34	19.8	0	95.7	
		7.5	47.0	48.9	53.6	7.29	20.8	10.4	97.0	0.3% CO in air
	2	6.6	48.9	40.0	55.9	7.40	19.2	0	96.1	
		6.9	47.6	43.9	54.2	7.35	19.5	9.5	98.5	0.3% CO in air
	3	6.7	46.4	43.8	52.8	7.35	20.0	0	96.3	
		6.7	46.6	44.9	53.1	7.33	19.9	3.4	97.7	0.2% CO in air

* Total CO_2 content of the arterial blood when pCO_2 is 40 mm.Hg.

† Blood drawn 10 minutes after the end of the rebreathing period.

‡ Blood drawn while the subject was breathing the mixture.

several occasions in H. C. and F. C., was within the usual range preceding and during the poisoning. This implies that no lactic acid or other fixed acid accumulated. Blood lactates, determined in most cases, were always low. The pCO_2 was elevated when the subjects had high CO concentrations. In all such cases the shift in pH was toward the acid side: this clearly depends on a depressed respiratory function. In two experiments

with moderate CO poisoning, the pCO_2 showed a slight decrease and the pH a slight increase.

TABLE 3

Respiratory, metabolic and circulatory effects of acute CO poisoning in man

SUBJECT	EXPERIMENT NO.	VENTILATION	O_2 USED	A-V O_2 DIFF.	CARDIAC OUTPUT	PULSE RATE	BLOOD PRESSURE	HbCO	REMARKS
		l. per min.	*cc. per min.*	*cc.*	*l. per min.*		*mm.*	*%*	
H. C.	1		221	56	3.9	61		0	
			218	42	5.1	76		41	Recovery
	2		221	56	3.9	58	100/70	0	
			246	42	5.8	87	120/80	35	Recovery
			246	46	5.3	78		32	Recovery
	3	6.6	239	55	4.3	62		0	
		6.9	240	40	5.9	98		42	
	4	7.1	240	55	4.3	68		0	
		6.8	261	43	5.9	103		52	
	5	7.1	240	62	3.8	64	96/72	0	
		6.8	242	59	4.1	70	102/66	16	
		6.9	238	40	5.8	81	104/72	32	
		6.8	242	43	5.6	92	106/64	45	
	6	5.9	230	61	3.7	62		0	
			230	59	3.8	62		0	
			230	60	3.8	62		0	
	7	6.0	225	55	4.0	61	124/84	0	
		5.9	219	53	4.0	70	116/78	23	
		5.8	231	45	5.1	80	118/78	43	
	8	5.9	230	55	4.1	65	108/76	0	
		5.9	220	53	4.0	72	104/72	25	
			228	50	4.5	76	110/74	30	
		5.4	228	45	4.9	82	107/68	39	
	9	6.1	235	62	3.7	64	102/74	0	
		6.0	226	50	4.5	76	108/74	31	
	10		228	55	4.0	66	106/74	0	
			218	42	5.1	79	106/72	33	
	11		221	53	4.1	67	104/72	0	
			221	54	4.0			0	
			223	38	5.7	76	108/74	32	

TABLE 3—*Concluded*

SUBJECT	EXPERIMENT NO.	VENTILATION	O_2 USED	A-V O_2 DIFF.	CARDIAC OUTPUT	PULSE RATE	BLOOD PRESSURE	HbCO	REMARKS
		l. per min.	*cc. per min.*	*cc.*	*l. per min.*		*mm.*	%	
F. C.	1	6.6	277	46	6.2	69		0	
		6.9	287	36	7.9	82		48	
	2	7.5	277	57	4.7	66		0	
		7.3	276	40	6.7	70		35	
	3	7.1	273	53	5.1	65	120/82	0	
		7.1	267	46	5.7	74	112/78	28	
				45	5.9		114/82	34	
		7.4	269	44	6.0	86	132/92	43	
	4	6.7	269	53	5.0	66	114/80	0	
		6.7	269	50	5.3	73	120/90	17	
		7.2	274	57	4.6	68	114/90	22	
		6.9	268	58	4.5	78	120/90	27	
	5	6.7	267	48	5.4	62	114/88	0	
		6.4	266	51	5.1	62	116/84	0	
		6.4	267	46	5.7	82	116/80	41	
	6	6.0	270	55	4.8	63	114/80	0	
		5.9		51	5.1	63	116/82	0	
		6.2	266	51	5.2	63	118/86	0	
		5.8	263	54	4.8	62	118/86	0	
S. H.	1		252	50	4.9	61	112/88	0	
			257	52	4.8	62	102/72	0	
			252	58	4.2	60	104/74	0	
	2		234	57	4.0	59	120/89	0	
			233	55	4.2	68	114/84	22	
			238	49	4.7	69	126/80	32	
			250	46	5.3	73	114/82	41	
	3		226	57	3.9	56	110/80	0	
			229	56	4.0	62	110/80	0	
			235	56	4.1	60		0	
	4	6.0	225	54	4.1	61	120/94	0	
		6.3	229	52	4.3	64	126/90	23	
		6.1	227			72	124/92	34	
		6.4	229	50	4.5	76	120/92	42	
	5	5.8	224	50	4.4	60	110/86	0	
		5.7	217	52	4.1	62	116/86	24	
		6.3	252	49	5.0	74	122/84	35	
		5.7	229	40	5.6	84	116/78	44	

In three subjects the average arterio-venous O_2 differences remained within the usual limits of day-to-day variations. Control determinations were made before each experiment. Three or more control determinations of the arterio-venous O_2 differences done during one day showed only slight changes. The A-V O_2 differences in the three subjects showed a

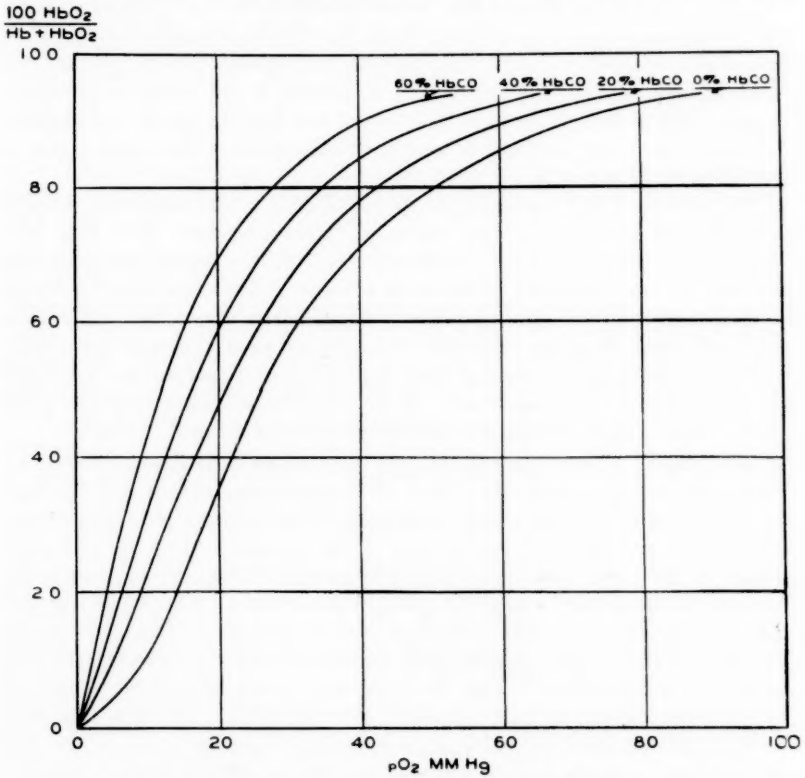

Fig. 1. Oxygen dissociation curves calculated according to Stadie and Martin in the presence of different percentages of HbCO.

moderate, progressive decrease when the concentration of CO in the blood was over 30 per cent. Therefore, the cardiac output increased, since the O_2 consumption remained unchanged. The increase in cardiac output was over 20 per cent, but not higher than 50 per cent, when the HbCO reached values of 40 per cent or over. With HbCO concentrations below 30 per cent, the changes in output were negligible. The pulse rate was increased when great amounts of HbCO were in the blood. The blood pressure also showed some elevation, although this was not a consistent finding.

Four oxygen dissociation curves, corresponding to 0, 20, 40 and 60 per cent HbCO, have been calculated according to Stadie and Martin (15) and are shown in figure 1. The values for the constants in the Stadie-Martin equation that most nearly accord with our dissociation curve for normal human blood are $N = -2.26$ and $\log K_{O_2} = -3.21$. Using these figures, the average venous pO_2 can be estimated with greater facility, as the following example will show (table 5). When the hemoglobin is 20 per cent saturated with CO, the available hemoglobin is 15.6 volumes per cent instead of 19.5. If the oxygen utilization is 5.3 volumes per cent, subtraction of this value from 15.6, and extending the value so obtained (10.3) to the corresponding oxygen dissociation curve, the venous pO_2 is found to be 27 mm.

The effect of CO hypoxemia on the excitability of the respiratory center was studied in two subjects (table 4). Concentrations of CO_2 in the inspired air varied from 1.8 to 5.2 per cent. The increase in ventilation as brought about by CO_2 is shown in terms of differences from the basal determination (2nd column). The third column shows the differences in ventilation obtained after the subject had been poisoned with CO. The two values for per cent of HbCO were obtained before and after the second period of CO_2 breathing. In each subject poisoned with CO, CO_2 produced less hyperventilation than in control experiments.

Discussion. Our observations and those of Asmussen and Chiodi (1) on man do not confirm the results of Haggard and Henderson (6) that dogs respond differently to CO while being poisoned than during recovery while breathing air. In contradiction to the findings of other investigators (6, 7) in both man and dog, no hyperpnea was evident, even though the concentration of HbCO in blood reached 52 per cent. Our results are in agreement with the clinical findings of Sayers et al. (13). Perfusion of the carotid body by Comroe and Schmidt (3) showed that as long as the arterial pO_2 remained sufficiently high no hyperpnea occurred despite reduction of oxygen content by CO. There is no reason for supposing that the arterial pO_2 is much reduced by CO poisoning. The elevation in arterial pCO_2 implies a slight reduction in alveolar pO_2, but this difference, perhaps 5 mm., would not reduce the arterial pO_2 below 75 or 80 mm. Hg. Since an increased pCO_2 coupled with a decreased pH ordinarily stimulates the respiratory center, the failure to observe hyperpnea in our experiments can only lead to the conclusion that the center was depressed. The failure of a given concentration of CO_2 in inspired air to induce as great an increase in ventilation in the hypoxia of CO poisoning as in the normal state is additional proof of depression. Dill and Zamchek (4) have shown that in a hypoxemia induced by breathing low oxygen, CO_2 has the opposite effect, i.e., an augmentation of the response to CO_2. These effects indicate that the chemoreceptors are essential in man to a potentiation of a hyperpnea when the arterial pO_2 is low.

There is much experimental evidence (14) that when the chemoreceptors of the carotid and aortic bodies of dogs are inactivated, hypoxemia depresses the respiratory center. So it appears that in man the depressant

TABLE 4

CO_2 hyperventilation in CO poisoning

SUBJECT	EXPERIMENT NO.	CO_2 PERCENTAGE	Δ_1*	Δ_2†	HbCO‡
			l. per min.	*l. per min.*	%
H. C.	1	1.79	2.2	1.5	30–26
	2	2.70	3.5	3.2	41–38
	3	3.35	5.4	4.1	33–29
	4	2.79	3.8	2.7	32–27
	5	4.87–5.09	10.7	10.1	34–26
	6	3.21	5.0	4.5	16–13
B. C.	1	3.18	4.4	4.3	0
	2	3.08	3.4	3.8	13–11
	3	3.10	4.0	3.0	33–29
	4	3.08	4.1	3.3	31–27
	5	5.22–5.03	13.5	12.2	32–24

* Δ_1 = difference between normal ventilation and hyperventilation induced by CO_2.

† Δ_2 = difference between ventilation breathing air and hyperventilation induced by CO_2 when subject is poisoned.

‡ Measured immediately before and after breathing CO_2-air mixtures.

TABLE 5

Venous pO_2 as estimated from O_2 dissociation curves of figure 1

	MAN AT REST WITHOUT CO	AT REST WITH 20% HbCO	AT REST WITH 40% HbCO	MAN IN MODERATE WORK WITHOUT CO	IN MODERATE WORK WITH 20% CO
Arterial HbO_2, vols. %	19.0	15.2	11.4	19.0	15.2
O_2 transport, vols. %	5.3	5.3	4.5	9.9*	9.3*
Venous HbO_2, vols. %	13.7	9.9	6.9	9.1	5.9
Venous saturation $\frac{100\ HbO_2}{Hb + HbO_2}$	68.5	62.0	57.5	45.5	36.9
Venous pO_2, mm. Hg	37.0	27.0	20.0	24.0	17.5

* As determined by Asmussen and Chiodi (1).

effect of CO on ventilation is similar to that produced by ordinary hypoxia in animals with denervated carotid and aortic bodies. The carotid and aortic bodies have an arterial flow so abundant that their pO_2 is essentially arterial. Since they depend for their stimulation upon the pO_2, they are not stimulated in the hypoxia of CO poisoning.

In agreement with most investigators, except Haggard and Henderson (6), a shift toward an acid pH was found. The alkali reserve (T_{40}) was not appreciably altered. This is to be expected in view of the unchanged blood lactate and the brief periods of hypoxia, which lasted slightly over an hour. The lack of a hyperpnea with an acid pH makes it difficult to believe that there is a simple relation between pH and ventilation.

Circulation. The arterio-venous O_2 differences decreased and the cardiac output increased after the CO concentration exceeded 30 per cent. The failure of Asmussen and Chiodi (1) to observe any significant increases during CO poisoning can be explained by their use of lower concentrations, ranging from 23 to 33 per cent. These effects of CO poisoning on the cardiac output are comparable to observations made during the anemias. Nielsen (12) followed the cardiac output during treatment of a patient suffering from pernicious anemia. The increase in arterio-venous O_2 difference occurred concomitantly with the increase in hemoglobin. Similar observations were made by Blalock and Harrison (2) on both acute and chronic types of anemia produced in dogs. An outgrowth of these and other experiments was the hypothesis that the capillary or tissue O_2 pressure might be an important factor in the regulation of the output of the heart. Our present experiments tend to confirm this hypothesis. The rôles played by the increased pulse rate and by the vasomotor reaction in giving an increased cardiac output still remain to be elucidated.

While the hypoxemia of CO poisoning affects respiration and circulation much like simple anemia, its internal action is more severe because of the greater reduction in the pO_2 within the tissues. Stadie and Martin (15) have shown that, as a result of the shift of the oxygen dissociation curve to the left during CO poisoning, there is a diminished partial pressure of O_2 in the tissues when compared to conditions in anemia.

Our measurements of the arterio-venous oxygen difference and of the cardiac output have enabled us to estimate the magnitude of this effect in various stages of CO poisoning. The calculated curves (fig. 1) illustrate this clearly.

SUMMARY

1. No hyperpnea was observable during rest in either dogs or men when subjected to acute and severe CO poisoning. The CO_2 combining capacity was unchanged, the arterial pCO_2 was increased, and accordingly the pH was shifted toward the acid side.

2. In severe CO poisoning the respiratory center was depressed.

3. The cardiac output showed no more than slight increases with HbCO saturations ranging up to 30 per cent. From that level up to 50 per cent HbCO the cardiac output increased as much as one-half.

4. The direct action on the respiratory center of the acute hypoxemia

produced by CO poisoning that is severe yet compatible with life is purely depressive in nature.

5. From the data given the oxygen tension in venous blood can be calculated for various levels of HbCO.

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THE ACTION OF TEMPERATURE ON THE EXCITABILITY, SPIKE HEIGHT AND CONFIGURATION, AND THE REFRACTORY PERIOD OBSERVED IN THE RESPONSES OF SINGLE MEDULLATED NERVE FIBERS

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Physiologists long have felt that in the modification of physiological activity resulting from temperature changes they possess a valuable means of acquiring information relative to the problem of tissue functioning and it is a tool that has frequently been employed to this end. In the case of medullated nerve fibers the interpretation of certain of the effects of temperature is complicated by the congregation in nerve trunks of fibers of different characteristics. To obviate this complication we have, for some years, been studying the responses of *single* axons and now are presenting observations we have made on the effects of temperature on aspects of nerve response that require axon spikes for their interpretation or that can be studied advantageously by means of single axon spikes.

METHODS IN GENERAL. The nerve used has been the branch of the peroneal supplying the medial aspect of digit IV of *Rana pipiens*. Preparations were discarded that did not contain a fiber of outstandingly high excitability, and observations were confined to the responses given by that fiber.

Stimuli were condenser discharges initiated by a gas discharge tube activated through a delay circuit by the sweep of the cathode ray tube. The condensers available ranged between 0.1 and 0.5μF. Shock strength was controlled potentiometrically by a Leeds-Northrup Kohlrausch slide wire with 100 scale divisions readable to 0.1 division.

Three methods for altering the temperature have been employed. In some of the experiments the changes involved the moist chamber and all of its contents. In other experiments the temperature of the nerve was altered locally where it was in contact with one or the other of the pair of stimulating electrodes. These were calomel half-cells so designed (Blair, 1938) that Ringer's solution could be circulated through them. The solution entered the upper orifice of the electrode (fig. 1) and issued via the horizontal, terminal, slit-like orifice which is crossed vertically by the

nerve. The rate of flow of the solution could be adjusted so as to give the half-cell the desired temperature. The flow was rather rapid and the temperature of the fluid was taken immediately before its entry into the electrode. When the temperature of the solution differed widely from that of the chamber the temperature at the orifice of the electrode may have differed by a fraction of a degree from the recorded temperature. The outflowing solution drained back away from the nerve along the lower edge of the electrode, after coming into contact with about 2 mm.

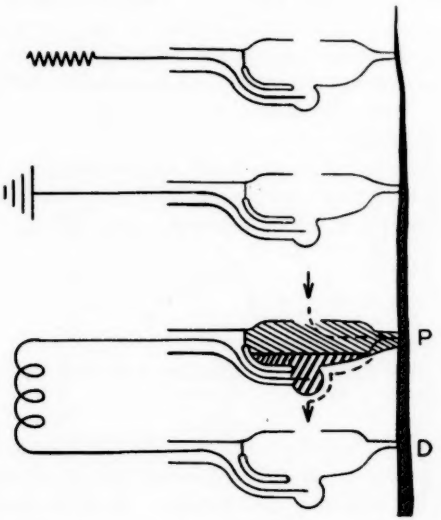

Fig. 1. Illustrating the arrangement of electrodes on nerve and method of changing temperature of nerve locally, in this case at the stimulated locus, *P*. The cross-hatching of electrode *P* indicates the contents of all electrodes: the lower level contains mercury and an emulsion of calomel and mercury; the upper level consists of Ringer solution. The solution that determines the local temperature takes the course indicated by the arrows and dotted line.

of the nerve's length immediately below the electrode. The flow produced not the slightest disturbance in contact, at least none that could be recognized when the electrode was the ground lead into the amplifier and amplification was maximal (about 2 mm. per μV). In this, the second of the cooling methods, the chamber was at room temperature while the nerve in contact with the half-cell was cooled. In the third method the chamber temperature was held constant at the low level while the temperature of the half-cell was raised to that of the room, when desired, by a continuous flow of the solution. The temperature of the chamber was taken at the level of, and close to, the nerve.

THE EFFECT OF TEMPERATURE ON EXCITABILITY. The electrical excitability of nerve as affected by temperature is a much studied subject. The earlier observers, using induction shocks as stimuli, concluded that cooling lowers excitability. Then it was reported by Gotch and Macdonald (1896) that when precautions are taken to circumvent the resistance changes of nerve caused by the temperature changes the result obtained upon cooling depends upon the "kind" of current employed: induction shocks disclosed a lowering of excitability, whereas constant current pulses longer than 5 msec. in duration, and condenser discharges, disclosed a raising of excitability. However, it was soon shown by Waller (1899) and by Lapicque (1907) that these differences are referable to the duration of the electrical currents employed as stimuli; currents of shorter duration decrease in effectiveness as the nerve is cooled, whereas currents of longer duration increase in effectiveness.

Method. Under the conditions of our experiments, the shocks employed by us for stimulation attain maximum height in less than 0.1 msec., and fall to one-third height in about 0.60 msec. (see fig. 2). The latter value

Fig. 2. Record (reproduced in photographed size) showing the configuration of the stimulating current. Time in 0.1 msec.

roughly approximates that of the spike at room temperature. If propagation and transmission of nerve impulses are by an electrical process the results obtained through the use of such a shock in temperature experiments should resemble somewhat those elicited by action potentials.

Results. Tested with these shocks, cooling a nerve invariably has raised its threshold. This result has not in our experience been altered by varying the resistance in the stimulating circuit, whether this was accomplished by adding resistance in series (up to as much as 1 megohm) or by varying the length of nerve subtended by the stimulating electrode through alteration of the position of the anode.

It can be shown, moreover, that the variation in the amount of current necessary to stimulate is the result primarily of the effect of temperature on excitability, rather than on resistance. Thus cooling only the region of the anode is without any appreciable effect on the threshold when, under similar conditions, cooling the cathode region produces the effect that is elicited through cooling the entire nerve.

The results of one of these experiments, carried out under the arrangements shown in figure 1, are collected in table 1. There it can be seen that upon cooling the nerve stretch that is in contact with the proximal

electrode, *P*, the threshold is raised when that electrode is the cathode, and not altered materially when that electrode is the anode. The results obtained when the distal electrode, *D*, is cooled are always very much less striking and they are variable. This variability is referable, we believe, to the relation the cooled stretch at *D* bears to the stretch of nerve polarized (*P-D*), the latter being mainly central to the former. However this may be, the results derived with *P* as the conditioned electrode are conclusive in themselves: any resistance change produced by the temperature change must be the same whether electrode *P* is cathode or anode.

TABLE 1

Effect of cathode or anode temperature on thresholds

RELATIVE THRESHOLDS	TEMPERATURE		CATHODE PROXIMAL (P) OR DISTAL (D)	CHANGE IN THRESHOLD $\frac{\text{WARM} - \text{COLD}}{\text{WARM}} \times 100$
	At cathode	At anode		
scale divisions	°C.	°C.		
592	22	22	D	
582	10	22	D	−1.7
567	22	22	D	+2.6
586	22	22	P	
575	22	10	P	−1.9
575	22	22	P	0
608	22	22	P	
710	10	22	P	+16.8
655	22	22	P	+9.2
705	10	22	P	+9.2
657	22	22	P	+8.8
622	22	22	D	
636	22	10	D	−0.9
636	22	22	D	0

Since the threshold changes only when it is cathode the change in threshold must be due primarily to the effect of the temperature on excitability.

In other experiments the temperature of the entire nerve was altered and determinations were made of the amount of current needed to stimulate with different interelectrode distances. Usually three electrodes were arranged on the nerve for stimulation. The electrode proximal with respect to the leads was cathode and in successive trials one or the other of the distal electrodes was made anode. In a crude way this procedure simulates that used by Rushton (1934) and by Cole and Hodgkin (1939) in determining separately the membrane and protoplasm resistance of nerve. They found that the slope of the resistance-versus-separation

curve is large for small electrode separations, but becomes smaller and finally constant as the separation increases; that "at 8 mm. the relation becomes constant with a gradient equal to the parallel resistance of the core and of the external fluid."

Under the conditions of our experiments the relation between electrode separation and the voltage necessary to stimulate becomes practically linear at about 5 mm. separation of the electrodes (see fig. 3). Of present

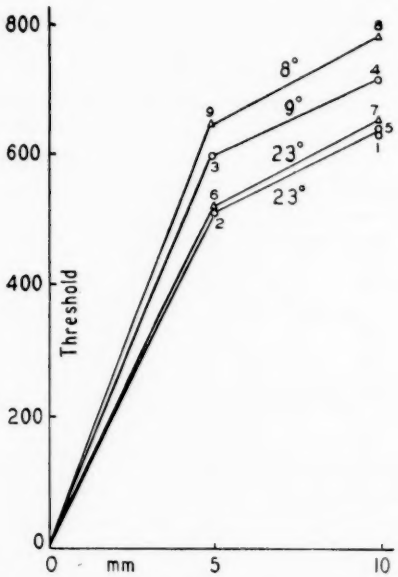

Fig. 3. Threshold *vs* interelectrode distance (resistance), the latter varied by altering position of anode of stimulating circuit. The sequence of the determinations and the femperatures are as indicated.

At the greater interelectrode distances the slopes of the curves are the same at the different temperatures.

interest, however, is the observation that beyond that separation, i.e., beyond 5 mm., the slopes of the curves are the same for different temperatures within the range tried, namely, 8° to 23°C. This result signifies that temperature alters excitability practically entirely through action on the membrane, or at least through action on the excited locus, and not perceptibly on the longitudinal resistance or on inactive nerve.

THE EFFECT OF TEMPERATURE ON SPIKE HEIGHT AND SHAPE. In a study of the effect of temperature, Gasser (1931) has employed as the "axon spike potential" the multifiber record obtained through stimulating the sciatic

nerve of the frog with shocks submaximal for the A elevation while leading about 4 mm. from the stimulating cathode. Though under these circumstances many axon spikes contribute to the record, he found no temporal dispersion even when conduction involved the entire length of the nerve provided threshold fibers alone were stimulated. To "eliminate the diphasic artifact" and associated difficulties he killed the nerve "as close as possible to the active lead" and "then the observations were made promptly before deterioration of the end of the nerve or partial reformation of the new plasma membrane. . . ". Under these circumstances he found that cold prolonged proportionally both the ascent and the descent of the spike and decreased its amplitude.

Method. We have met in another way the conditions specified by Gasser. In our final experiments we have used only those preparations the most excitable fiber of which transmitted impulses the entire interlead stretch, that is to say, only those yielding an action potential which initially was *completely* diphasic. Then the record was made monophasic by circulating isotonic KCl through the electrode acting as the distal lead, and the effect determined of changing the temperature of the nerve under the proximal lead, the rest of the nerve remaining at the temperature of the chamber (room temperature).

Failure to take these precautions accounts, we believe, for some variability encountered in the earlier experiments (to be referred to below). If initially the record happens to be monophasic, or nearly so, because of some fortuitous injury between leads, it tends with the lapse of time to become somewhat, and often unrecognizably, diphasic. Moreover, if this injured locus between leads happened to lie outside the range of action of the KCl applied through the distal electrode, the application of the KCl would not eliminate that diphasicity, and it might then go wholly unrecognized.

In determining the effect of temperature on the height and configuration of the spike of a single fiber it is essential that the recording mechanism be sufficiently quick to record the spike accurately. To test our mechanism a rectangular current was passed through the digital nerve via two calomel half-cells acting as electrodes. The lead into the amplifier was via two other calomel half-cells placed, about 6 mm. apart, on the nerve between those through which the current was applied. The recorded deflection attained 95 per cent of maximum in 0.16 msec. Since the fastest spike recorded in this investigation has had a time to maximum of 0.27 msec., and since the time to maximum at the higher temperature usually has been longer than 0.4 msec. and at the lower temperatures often as long as 0.8 msec., it is certain that the spikes have not been measurably distorted in the recording.

Results. Employing the precautions described above, cold invariably

has *prolonged the descent* of the spike *more than the ascent* and has *increased the height* of the spike; consequently it has increased enormously the area of the spike.

A typical experiment may be described. It is illustrated in figure 4, and the data are included in table 2 (expt. 5/23). Stimulation is indicated by the shock artifact. The interlead distance was 1.25 cm., which happens in this case to be the conducting distance, also. A and B are pictures obtained when the recording was frankly diphasic, A while the whole nerve was at the uniform temperature of 26°C., B while the nerve in the immediate vicinity of the proximal lead electrode was cooled to 8°C. The

TABLE 2

Effect of temperature on spike configuration and height

EXPERIMENT	INTERLEAD DISTANCE	CONDUCTION RATE AT GIVEN TEMPERATURE	TEMPERATURE	SPIKE ASCENT		SPIKE DESCENT		HEIGHT (RELATIVE)
				Duration	Ratio	Duration	Ratio	
	mm.	*M/sec.*	*°C.*					
5/15	11		25.1	0.35		0.50		100
			9.0	0.58	1.66	1.70	3.40	116
			24.5	0.39	1.49	0.52	3.27	100
	4		24.9	0.38		0.56		100
			9.0	0.58	1.53	1.06	1.89	110
			25.0	0.43	1.35	0.61	1.79	102
5/22	16		8	0.68–1.15		3.57–4.9		107–121
		20	25	0.52	1.3–2.21	0.755	4.73–6.49	100
5/23	12.5		8	0.55±		2.05		128
		26	26	0.39±	1.41±	0.50±	4.1±	100
5/24	16		8	0.62±		3.85		133
		27.6	26	0.37±	1.68±	0.51	7.54	100

presence of a shock artifact introduces some uncertainty into the location of the starts of the spikes. Accepting as the starts points selected somewhat arbitrarily, and allowing for some latency of response, the conduction time is found to be less than 0.50 msec. and the conduction rate, therefore, faster than 25 m.p.s., these with the entire nerve at 26°C. The interlead time, therefore, was slightly less than 0.5 msec. Since the time to maximum of the "warm" spike is 0.32 msec., its crest must have recorded at the proximal lead before the spike arrived at the distal lead; its ascending phase consequently must have recorded without deformation. This conclusion is confirmed by the fact that the height of the spike recorded at the proximal lead is not increased when, as will be explained below, it is pre-

vented from arriving at the distal lead (see fig. 4C). Then the temperature of the nerve at the proximal lead was lowered to 8°C. while the rest of the nerve remained at 26°C. The chief obvious result (B) is the prolongation of activity at the proximal lead; it now outlasts the process at the distal lead, so that the spike, as it affects the distal lead appears as a notch in the spike recorded at the proximal lead. Other minor changes referable to the temperature change are *a*, slight latening of the spike, and *b*, of the apex of the second phase, and *c*, broadening of the first elevation. The first two of these effects are manifestations of prolongation of conduction time,—they are slight because only a few millimeters, at the most, of the nerve's length are subjected to the temperature change.

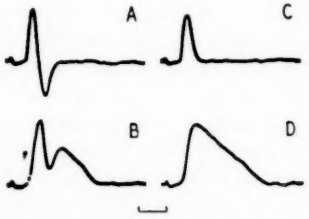

Fig. 4. Records (reproduced in photographed size) showing the effect of temperature on spike configuration (expt. 5/23, table 2).

A, completely diphasic; temperature of proximal lead 26°, of distal lead 26°. B, completely diphasic; temperature of proximal lead 8°, of distal lead 26°. C, completely monophasic; temperature of proximal lead 26°, of distal lead 26°. D, completely monophasic; temperature of proximal lead 8°, of distal lead 26°.

The small initial peak is the beginning of the shock artifact. Conduction distance = 1.25 cm. The bracket subtends 1 msec.

Having thus demonstrated that the fiber was conducting its full length, the recording now was made monophasic by the method described above, and records were obtained at the same two temperatures. They are reproduced as C and D. Except for the absence of the second phase these records match perfectly those of the first set: cooling prolongs the descent of the spike much more than the ascent and increases the height of the spike.

The data derived in this way from four preparations are included in table 2. Since temperature coefficients of nerve activities are dependent in a measure on the level of the subtended temperatures (Gasser, 1931), we refrain from presenting our data in terms of coefficients. It will suffice to say that in the range within which we have worked, the prolongation of the descent by cold always has been more than twice that of the ascent; in one case it was 4.5 times as great.

There are six other experiments, antedating those listed in the table, in

which initial complete diphasicity was not checked. In some of those experiments the stimulus was applied to the nerve at the proximal lead, in others at a remote point. In some the temperature of the entire chamber (of the entire preparation) was changed, in others the temperature at the proximal lead only. In some the nerve was killed by crushing, in others by the application of potassium chloride via the distal lead electrode. In all save one preparation cold increased the duration of the descent more than that of the ascent. In the case of the exception the ratio of the increase of the descent was 1.1, of the ascent 1.31; in other words, the ratios were alike within the limit of error. In view of the fact that there may have been a degree of concealed diphasicity in all of these earlier records we are not assigning any significance to this single exception to the rule.

Cold increased the height of the spike in all cases.

Cold is known to greatly enhance reflex activity of the spinal cord (see for example, Grundfest, 1941). Since cold increases spike height and duration these might be factors, along with others, such as the diminution of accommodation by cold, contributing to the increased activity of the cord.

THE RELATION OF THE ABSOLUTELY REFRACTORY PERIOD TO SPIKE DURATION AT DIFFERENT TEMPERATURES. *Method.* The ideal method for the determination of this relationship would be to compare the duration of the spike at the lead with the refractory period at thatl ocus. It requires, however, that there be at the lead only one fiber that will respond to a stimulus that is over 5 times as strong as the threshold of that fiber. Since this not a practicable condition, we have resorted to the method, previously employed by us (Blair and Erlanger, 1933), of stimulating one of the trunks of origin of the sciatic and recording in the phalangeal branch the spike of an adequate single fiber, when there happened to be one. The objection to the method lies in the fact, mentioned above, that the refractory period determined at one locus is compared with the spike duration determined at another.

Results. Gasser (1931), employing a multifiber response, found that "the temperature coefficient for the absolutely refractory phase is very close to that of the spike, but tends to be slightly larger, particularly in the lower temperature range."

Owing to the inadequacy of available methods, explained above, and since qualitatively we have obtained the same result as Gasser, we have carried through only two determinations. The results of one of these are plotted in figure 5. There it is seen that at the high temperature the discrepancy is narrow, that it is wide at the lower temperatures.

Here reference should be made to an observation, made in another place (Blair and Erlanger, 1933; also Erlanger in Erlanger and Gasser, 1937, p. 48), which casts some doubt on the value of these comparative determinations of the absolutely refractory period. In those determinations it

frequently happened, when dealing with fibers of low excitability, that the values for the absolutely refractory period were larger than those for the relatively refractory period. Such a state of affairs is, of course, wholly anomalous, and we consequently regarded it as an artifact produced by the very strong shocks needed under those circumstances. It still is possible, therefore, that the prevailing view (Adrian, 1914) is correct, that spike duration and absolutely refractory period actually are coterminate, though this remains to be demonstrated.

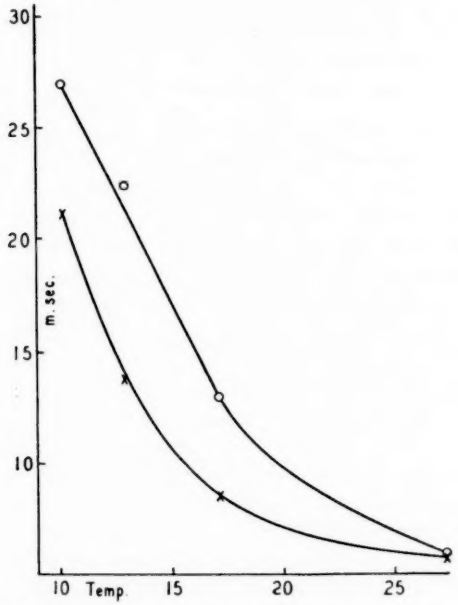

Fig. 5. Graph of a determination of the effect of temperature on the absolutely refractory period (circles) and spike duration (crosses).

SUMMARY

In observations made on the responses of single medullated fibers of the frog it is found that:

a. Cold increases the current strength required to stimulate, mainly through the effect of temperature on excitability, the effect on resistance being inappreciable,—

b. Prolongs both the ascent and the descent of the spike, but the descent *much* more than the ascent, and,—

c. Increases the height of the spike.

Reference is made to the possible relation of these effects to the enhancement of reflex activity by cold.

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A STUDY OF THE SPONTANEOUS OSCILLATIONS IN THE EXCITABILITY OF NERVE FIBERS, WITH SPECIAL REFERENCE TO THE ACTION OF STRYCHNINE

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When the most excitable fiber of a frog's nerve is stimulated with threshold shocks of uniform strength at relatively slow and regular rates not all the stimuli initiate conducted spikes. The range of shock strength between one that just fails ever to initiate any spikes and one that just suffices always to stimulate covered in our previous work about 2 per cent of the stimulating voltage. Associated with this "spontaneous" fluctuation in excitability there is a fluctuation in response latency. When two or more fibers happen to have about the same thresholds and are responding to the same threshold stimuli their excitabilities and latencies fluctuate quite independently one of the other, an observation which proves that the phenomenon is not referable to unrecognized fluctuations in the strength of the stimulus (Blair and Erlanger, 1933).

All of these observations have been confirmed by Pecher (1936; 1937; 1939). In addition, Pecher has concluded, on the basis of a statistical treatment of the subject, that the fluctuations in excitability are completely random, with the qualification that when the rate of stimulation is fast enough the exteriorization of the latency fluctuations may be modified by the resulting disbandment and facilitation (see Erlanger and Blair, 1940). Failing to obtain any clue as to their nature, Pecher concluded that the spontaneous variations are molecular in origin.

On the basis of this inference one would expect exposure of the nerve to conditions which alter molecular perturbability to change correspondingly the range of the spontaneous alterations in excitability and that conditions which change the nerve's excitability without altering molecular perturbability would be without influence on the spontaneous fluctuations. Of influences belonging in the first category temperature might be expected to have a definite effect; and in the second category one would expect to find local anode or cathode polarization and local treatment of the nerve with sodium citrate. This paper deals with the effects of these agents, and also of strychnine, on the amplitude of the spontaneous changes in excitability.

METHODS IN GENERAL. Observations have been made on the responses of single nerve fibers, employing the technique described in the preceding paper (Schoepfle and Erlanger, 1941). Again, the nerve used has been the branch of the peroneal supplying the medial aspect of digit IV of *Rana pipiens*; and all preparations were discarded that did not contain a fiber of outstandingly high excitability and the observations were confined to the responses given by that fiber. Usable preparations are obtained in about 30 per cent of the trials. The fineness of the digital nerve renders it particularly favorable for observations on the action of chemical agents, owing to the promptness with which their action becomes manifest (Erlanger and Blair, 1938).

Since the excitability of a fiber is constantly shifting in random fashion, though between certain limits, a single determination of the range is a time-consuming process, sometimes requiring several hundred stimulations (see below); and since under some of the imposed conditions it was difficult to hold the nerve in a constant state for any great length of time, determination of the parameters of strength-duration curves was out of the question. Consequently, for test stimuli we have resorted to shocks with a temporal configuration very roughly approximating that of a nerve action potential (see preceding paper).

The stimulation rate was constant during any set of observations, and usually about 1 per second. At the start of each set of determinations the sliding contact of the potential divider was set in such a position that the maximum sensitivity would be available that was compatible with the anticipated alterations in threshold, and then the resistance in the end coils was adjusted so that the selected position of the sliding contact was the threshold position. If, during the course of an experiment the amount of current necessary to stimulate happened to increase to the point where it exceeded the limits of the instrument thus set, all preceding data had to be discarded and the observations started again with another initial setting of the sliding contact.

Since the spontaneous changes in excitability are relatively small it was essential to have adequately constant stimuli. Determinations of the variability of the stimulating voltage were made at frequent intervals during the course of each experiment, employing a gas discharge tube activated by the stimulus discharging through a loud speaker as indicator. The full voltage varied less than 0.66 per cent and in some cases as little as 0.24 per cent. Since the *full* range of the spontaneous oscillations, when determined, has covered about 8 per cent of the threshold, it follows that variations of less than 8.3 per cent are, due to this source alone, within the range of error of the method $\left(\frac{0.66}{8} \times 100\right)$. As a matter of fact, we do not

regard as significant any induced change that is less than 20 per cent, unless the sign of the change is constant in successive experiments.[1]

It is not, however, practicable to employ as a measure the *full* range of the oscillations. The reason for this can be made clear by considering the configuration of the curve of the relation between the per cent of the stimuli that elicit responses and the strength of current in per cent of the absolute threshold, the absolute threshold being the current strength that just fails ever to stimulate. Such a curve is reproduced in figure 1. In this case the range of shock strength between one that elicits no responses and one that never fails to stimulate amounts roughly to 8 or 9 per cent of the threshold. Since the curve is S-shaped, the extremes of the range are

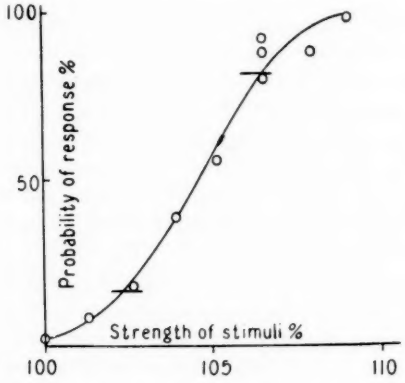

Fig. 1. Graph showing the relation between the probability of a response in per cent and the strength of the stimulus in per cent of the absolute threshold, assigning a value of 100 to the strength that just fails ever to stimulate. The horizontal lines subtend the part of the range measured, i.e., from 1 response to 6 shocks to 5 responses to 6 shocks.

difficult to determine experimentally. In the effort to minimize this difficulty we have adopted as end points the shock strength which elicits upon the one hand an average of one response in six stimulations, and upon the other five responses in six stimulations. Even so, the randomness of the variations necessitates long series of stimulations for each determination. In the present instance the range between one response in six stimu-

[1] Alterations in configuration and duration of currents also modify their effectiveness as stimuli. These have not been controlled. In view of the fact, however, that when two or more fibers of the same threshold are responding to threshold stimulation there is no correlation between their responses, it may be justifiable to assume that shape changes of the shocks have not qualified the results obtained.

lations and five responses in six stimulations amounts to slightly over 4 per cent of the threshold stimulation strength. This is a relatively high "normal" value, as may be seen in table 1 where the usual normals, as defined here and in the remainder of the paper, lie between about 2 and 4 per cent.

The relative amplitudes of the oscillations under the different conditions of an experiment are given in each case by the two limiting readings, but only when the nerve's resistance is not altered by those conditions. Any alteration of this resistance would cause an inversely proportional change in the value of the divisions of the potential divider. That is to say, if the resistance alone were to change, and not the oscillations in excitability, the "oscillation" ranges as read would be proportional to the "threshold" readings.

Though this assertion is an obvious requirement of Ohm's law when considered in relation to the fact that it is current and not voltage that stimulates, experimental verification, nevertheless, seemed desirable. This was accomplished by determining the effect of varying the interelectrode resistance by shifting the position of the anode of the stimulating current along the nerve. To cite the results of such a determination, increasing the interelectrode distance from 9 mm. to 27 mm. increased the average "threshold" readings given by the potential divider from 553 to 807, and the average of the oscillations from 18 to 27. The ratios of the oscillations to the "thresholds" are 0.0307 and 0.0299, that is to say, they are the same.

Since, then, the oscillation amplitudes as read will be proportional to any intercurrent resistance changes, and are proportional also to the real excitability range, the results of any imposed condition on oscillation amplitude require for their complete interpretation data on resistance. We have not, however, measured resistances and therefore must resort to recorded information, and this is scant, in discussing our results. It will be necessary to assume that all changes in oscillations as read on the potential divider actually are due to changes in excitability unless there are reasons for believing that the interposed conditions have altered in a known direction the nerve's resistance.

ANODE AND CATHODE POLARIZATION. *Methods.* The threshold of the nerve at the cathode of the stimulated locus has been raised or lowered by means of local anode or cathode polarization, respectively. This was accomplished by methods described elsewhere (Erlanger and Blair, 1936).

Results. Anode polarization (table 1, expts. 11/7 a.m., a and b, and p.m., 11/15) of an intensity that raises the threshold as much as 70 per cent has not altered the amplitude of the oscillation by an amount that significantly exceeds the probable experimental error, excepting one case (11/15) where the oscillations diminished 29 per cent. Moreover, the sign of the alteration has not been constant from experiment to experiment.

Cathode polarization (expts. 11/7 p.m., 11/15) that lowers the threshold

TABLE 1

Data on thresholds and oscillation amplitudes

EXPERIMENT	PROCEDURE	LOWER THRESHOLD	CHANGE OF THRESHOLD	OSCILLATION AMPLITUDE	OSCILLATION RANGE RELATIVE TO THRESHOLD	CHANGE IN OSCILLATION AMPLITUDE	REMARKS
		scale div.	*per cent*	*scale div.*	*per cent*	*per cent*	
11/7-a	Normal	590		22.0	3.7		
a.m.	An. pol.	838	+42.8	17.2		−21.8	
11/7-b	Normal	419		10.0	2.4		Same preparation, but another fiber
	An. pol.	728	+73.7	11.5		+10.5	
11/7	Normal	392		8.7	2.2		
p.m.	Cath. pol.	274	−30.1	9.0		+3.4	
11/15	Cath. pol.	435	−20.8	25.0		+19.0	
	An. pol.	637	+21.6	22.2		+5.7	
	Normal	524		21.0	4.0		
	An. pol.	749	+43.0	21.0		0	Polarization strength increased
	Cath. pol.	304	−42.0	13.0		−38.1	
	Cath. pol.	313	−40.3	16.5		−21.4	
	An. pol.	740	+41.2	14.8		−29.5	
11/25	Normal	743		25.0	3.4		
a.m.	0.7 NaCl	657	−10.4	22.3		−10.8	
	0.7 NaCl	605	−18.6	25.0		0	At end of about 30 min.
	Na citrate	250–170*	−66.2 −77.2	25–20–			About 40 min. treatment; at end the small fibers were repeating
p.m.	Normal	618		20.0	3.2		
	Na citrate	190–127	−69.2 −79.4	20.0±		0±	Terminated because fiber became inexcitable
11/28	Normal	918		25.0	2.7		
	Na citrate	515–450	−43.9 −51.0	28.7		+14.8	Readings started after 60 min. and continued for 44 min.
	Washed	600	−34.6	26.0		+4.0	
	Normal	875		22.5	2.6		Another part of nerve. New setting of potential divider
	Na citrate	342–310	−60.9 −64.6	41.6		+85.0	Readings started after 57 min. and continued for 60 min.
	Washed	610–700	−30.3 −20.0	29.6		+31.6	Readings begun after 25 min.

* Two values thus recorded in this column signify that the threshold changed through that range during the observation. This happened almost invariably during citration and occasionally during low temperature observations. Moreover, the return toward normal after citration is so slow that the "normal" thresholds at that stage always have been below the initial normal. When conditions could not be kept constant the order of the readings (high threshold-low threshold) was alternated throughout the period of observation.

TABLE 1—*Continued*

EXPERIMENT	PROCEDURE	LOWER THRESHOLD	CHANGE OF THRESHOLD	OSCILLATION AMPLITUDE	OSCILLATION RANGE RELATIVE TO THRESHOLD	CHANGE IN OSCILLATION AMPLITUDE	REMARKS
		scale div.	*per cent*	*scale div.*	*per cent*	*per cent*	
12/4	Normal	782		15.5	2.0		
	Citrate	350–200	−55.2	16.4		+ 5.8	Readings begun after 40 min. and continued for 30 min.
			−74.4				
	Washed	360–425	−54.0	16.2		+4.5	After 22 min.
			−45.7				
6/16	Normal	622		20.0	3.2		
	Citrate	215	−65.4	20.3		+1.5	
			−59.8				
	Washed	535		23.3		−9.1	After 30 min.
12/5 a.m.	23°	492		15.5	3.2		Temperature of entire chamber altered
	13.2–11.8°	905–942	+102	15–42.5		−3.2	Observations over a period of about 30 min.
			+91.4			+174	
			+103			+12.8	
	23°	444	+112	13.3		+219	
p.m.	23°	407		12.7	3.1		During and after the cooling period of this exp. there was obviously a progressive decline in oscillation amplitude. All comparisons are with the first room temperature readings
	12.8–11.0°	615–655	+51.1	19.0		+49.6	Stim. rate 65/min.
			+61.0				
	11.2–11.7°	620–645	+52.2	15.0		+18.1	Stim. rate 30/min.
			+58.5				
	11.7°	622	+52.8	13.0		+2.4	Stim. rate 65/min.
	21.0–22.5°	388–413		11.0			Stim. rate 65/min.
12/18	23.5°	437		15.2	3.5		Temperature of entire chamber altered
	13.4–10.0°	540–605	+23.6	24.1		+58.5	
			+38.5			+119	
			+24.2				
			+39.1				
	21.0–21.8°	435		11.0			
1/21	21.8°	593		17.2	2.9		Chamber maintained at room temperature; cathode temperature altered
	13.0°	754	+27.2	23.7		+37.8	

TABLE 1—*Concluded*

EXPERIMEN	PROCEDURE	LOWER THRESHOLD	CHANGE OF THRESHOLD	OSCILLATION AMPLITUDE	OSCILLATION RANGE RELATIVE TO THRESHOLD	CHANGE IN OSCILLATION AMPLITUDE	REMARKS
		scale div.	*per cent*	*scale div.*	*per cent*	*per cent*	
1/23	12.9°	801	+26.4	16.0		+1.9	Chamber maintained at low temperature; cathode temp. altered. Experiment proceeded at an unusually slow rate
	19.3–20.5°	605–663 (634)		15.7	2.5		
1/30	7.8°	504		21.0			Chamber maintained at low temperature; cathode temp. altered
	19.0°	366	+37.7	14.8	4.0	+41.9	
			+59.3			+41.9	
	7.8°	585		21.0			
4/28	Normal	572		15.7	2.7		
	Strych.	782	+36.7	55.0		+255	After 30 min.
	1:1000	753	+31.7	60.0		+282	After 60 min.
	Washed	430		15.0			After 60 min.
	Normal	492		15.0	3.1		
	Strych. 1:1000	650	+32.1	70.0		+367	
	Washed	527		22.5			New setting of potential divider
	Normal	528		21.7	4.1		
	Strych.	707	+33.9	90–55		+315	Two readings; time interval not mentioned
	1:1000					+153	
4/29	Normal	533		10.0	1.8		
	Strych.	644	+17.7	28.0		+180	After 60 min.
	1:100,000	648	+18.6	27.3		+173	After 105 min.
	Washed	730?		12.7			After 60 min.
5/4	Normal	528		11.8	2.2		
	Strych.	602	+14.0	58.3		+394	After 60 min.
	1:000,000		+12.3			+359	
	Washed	537		12.7			After 90 min.
6/27	Normal	600		10.0	1.7		
	Strych.	810	+35.0	15.0		+50	After 60 min.
	1:100,000						
	1:10,000	825	+37.5	20.0		+100	After 45 min.
				20.0+			
	Normal	580			1.7		After 60 min.

as much as 42 per cent likewise has failed to alter the oscillation amplitude by more than the experimental error, again with one exception (in the same experiment as the one mentioned above), in which the amplitude was diminished 38 per cent. And, as happened with anode polarization, the sign of the change produced by cathode polarization has not been constant.

Discussion. The cause of the variability of these results is not as yet clear. The possibility that uncontrolled demarcation currents may have been a factor is suggested by a comparable difficulty encountered in another connection (Blair and Erlanger, 1940), one that was eventually controlled by confining observations to portions of the sciatic nerve that were removed at least 10 mm. from any injured region. That span cannot be assuredly obtained in the digital nerve.

The increase and the decrease in the number of scale divisions needed to stimulate when nerve is polarized anodally or cathodally is usually ascribed to reduction or increase, respectively, in excitability. But Cole (personal communication) informs us that the steady state "resistance" of the squid membrane is increased at the anode and decreased at the cathode. It is possible, therefore, that our oscillation readings are too high while the nerve is anodally polarized, and too low while it is cathodally polarized. It is on account of this uncertainty that observations on the effects of polarization have not been multiplied. For the present we can conclude only that the observed changes in oscillation amplitude determined by polarization are within the limits of error of the method.

THE EFFECT OF SODIUM CHLORIDE. A single experiment (11/25), in which the nerve was treated with isotonic sodium chloride, gave negative results. The solution was applied by the method referred to below.

THE EFFECT OF SODIUM CITRATE. *Methods.* The solution used consisted of 90 cc. of 0.7 per cent sodium chloride and 10 cc. of 3.2 per cent sodium citrate. To apply it to the nerve, it was circulated slowly and steadily through the calomel half-cell acting as the cathode of the stimulating circuit (see preceding paper). It came into contact with the nerve in the immediate vicinity of the stimulating cathode only.

Results. In this concentration the citrate solution may cause the smaller fibers of the preparation to discharge spontaneously, but not the large fiber that is under observation. The threshold of this fiber may be lowered as much as 80 per cent by the treatment. The amplitude of the spontaneous oscillations is not changed (11/25 a.m. and p.m., 6/16) or is increased (11/28, 12/4). In one instance only (11/28) was the increase definitely greater than the experimental error.

Discussion. Though the citrate solution may increase the amplitude of the oscillations, the effect cannot be regarded as striking. What effect, if any, citrate exerts on the ohmic resistance of nerve does not seem to be known. The finding of Höber et al. (1939) that it does not alter resting potential may perhaps be taken to signify that it does not alter resistance.

On this basis one may be justified in concluding that if citrate solutions alter the oscillation amplitude at all, they increase it.

THE EFFECT OF TEMPERATURE. *Methods.* The temperature of the nerve has been lowered (or raised) by all of the methods described in the preceding paper.

Results. There are six sets of observations. In all cooling increased the oscillation amplitude. In five of the sets (12/50 a.m. and p.m., 12/18, 1/21, 1/30) the increase exceeded the limit of error; in one case (1/23) it was scarcely perceptible.

Discussion. Cooling the nerve increases quite decidedly the applied voltage required to stimulate. In the preceding paper evidence was presented indicating that this increase is due primarily to the lowering of *excitability* by cold, not to an increase in resistance. One must conclude, therefore, that the increase in the amplitude of the spontaneous oscillations elicited by cold is real.

Cold is known to increase reflex activity of the spinal cord (Grundfest, 1941). How an increase in oscillation amplitude might act to produce this effect will be considered under the discussion of the central action of strychnine. In the preceding paper it was found that cooling nerve increases the height of the spike and prolongs it, and it was there suggested that these changes might have the effect of increasing the efficacy of the spikes as stimuli. Both of these effects of cold, the effect on the spike and the effect on the oscillations, might therefore contribute to the reaction of the cord to cold.

THE EFFECT OF STRYCHNINE. *Methods.* Purified strychnine sulphate was dissolved in Ringer's solution free of HCO_3 and PO_4, and, thus dissolved, was applied to the stimulated locus by circulating it through the half-cell acting as the cathode. With one exception the concentrations employed have been 1:1,000,000, 1:100,000 and 1:1,000.

Results. The weakest of these solutions was without demonstrable effect. This result gives assurance that the modified Ringer's solution does not contribute to the results to be described.

Striking reactions were obtained with the other concentrations of strychnine. As may be seen in the table, the 1:100,000 solution (expt. 4/29, 5/4, 6/27) raises the threshold slightly, 14 to 35 per cent, but increases enormously the oscillation amplitude—as much as 394 per cent. This maximum is attained within one hour and is entirely reversible after one and one-half hours.

Similar effects were obtained with the 1:1,000 solution (expt. 4/28, *ter*). In this concentration the strychnine raises the threshold somewhat more, 32 to 37 per cent, but the order of magnitude of the increase in oscillation amplitude remains the same. In one case this solution annulled excitability irreversibly after 105 minutes.

The action of the strychnine solution on the spike requires mention here.

Treatment with the 1:100,000 solution long enough to bring out the effects described above has had no obvious effect on the amplitude or duration of the spike. There is but one observation with a 1:10,000 solution: the threshold was raised 37 per cent and the oscillations 100 per cent, at a time when there was no measurable effect on the spike. The 1:1,000 solution can produce its maximum effect on the oscillation amplitude at a time when the spike is altered only slightly; on the other hand, the oscillation amplitude may still be high at a time when the height of the spike is markedly reduced and its breadth increased (see fig. 2). The descent of the spike then is prolonged much more than its ascent.

Discussion. Changes in the configuration of the spike due to treatment with strychnine have not been alike in the experience of different investigators. Peugnet and Coppée (Coppée, 1936; Peugnet, 1936) state that it is modified by a 1:2,000,000 solution. The duration of treatment necessary to bring about this modification is not given, but they picture the effect of treatment with a 1:50,000 solution for 138 minutes and with a

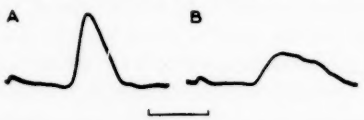

Fig. 2. Strychnine action on spike (expt. 4/28). A,normal. B, after 1:1,000 strychnine: ascent prolonged 33 per cent, descent 95 per cent; threshold raised 33 per cent; oscillations increased more than 300 per cent. Weaker solutions do not alter the spike.

The bracket subtends 1 msec.

1:10,000 solution for 20 minutes. The former modified the spike only slightly, the latter markedly. Heinbecker and Bartley (1939) state that the only effect of strychnine on the spike is to decrease its height. In our experience, as stated above, an obvious change in the spike has been obtained only with the 1:1,000 solution. Peugnet and Coppée found that the rheobase is lowered by concentrations of less than 1:1,000,000 and raised by greater concentrations. By our method of testing, strengths of 1:100,000 and 1:1,000 have raised the threshold.

Since, in our experiments, strychnine raised the threshold but slightly (not over 37 per cent), only a very small fraction, at the most, of the increase in the oscillation values can be referred to an effect of strychnine on nerve resistance. It would, however, be surprising if strychnine exerted any effect whatever on nerve resistance. There is every reason for believing, therefore, that practically the whole of the increase in oscillation amplitude produced by this agent is real.

The fact that different investigators fail to get the same change in the spike through exposure of the nerve to strychnine can be accounted for

only on the basis of unrecognized differences in technique or materials. The essential observation, from the present standpoint, is that the concentration, namely, 1:100,000, which in our hands has not obviously altered the spike, elicits oscillations that are as wide as any that have been noted with any concentration. And since the threshold is raised but little (it may be by only 12 per cent) at a time when the 1:100,000 solution is eliciting these wide oscillations, it seems quite possible (particularly in view of Peugnet and Coppée's observation that very dilute solutions *lower* the threshold) that there is a dilution at which oscillations still are at their maximum while the threshold is normal or lower than normal.

The striking increase in the amplitude of the spontaneous oscillations in excitability of nerve fibers that is elicited by strychnine suggests that this phenomenon may be a factor in the production of the central action of that alkaloid. Since, however, the oscillation swing has at no time carried the threshold, raised by the action of strychnine, below its normal level, it is obvious that the strychnine oscillations could not, without some collateral action, account for strychnine convulsions. However, one is reminded in this connection of the fact that strychnine convulsions ordinarily do not occur in the absence of a play of afferent impulses on the strychninized centers; that, moreover, the topical application of strychnine to both the dorsal and the ventral regions of the cord is requisite to the induction of tetanus (Dusser de Barenne, 1911). Is it not possible, therefore, that it is the lowering of the central threshold by afferent impulses that permits the spontaneous oscillations in excitability, increased in amplitude by the strychnine, to attain that threshold and so to produce the convulsions?

The electrical activity of the cord during strychnine tetanus, recently described by Bremer in a preliminary report (1941), prompts another hypothesis utilizing the effect produced by strychnine on the amplitude of the oscillation in excitability. "Everything suggests," Bremer writes, "that this neuronal synergy (of the cord) is effected by an intercellular action, truly electrical in nature, which spreads from an occasional focus with the speed of an explosion and synchronizes, over wide areas, the pulsations of autorhythmic elements possessing nearly identical individual frequencies." It may be that the autorhythm premised by Bremer is the rhythm of our spontaneous oscillations in excitability, increased in amplitude by the action of strychnine—that the rate of the "tetanic waves," 30–10 per second, is the rate of those oscillations. It is more likely, however, since this is the usual rate of discharge of anterior horn cells, that the "occasional focus" is a group of anterior horn cells, reflexly activated. The Bremer picture, then, would be reproduced by stimulation, through electrotonic spread from those cells, of neighboring cells which momentarily found themselves in the low threshold phase of their excitability oscillations, enhanced in amplitude by the action of the strychnine. This

stimulation would occur without synaptic delay, and so would be accounted for the "spread" of the process through the cord "with the speed of an explosion."

Heinbecker and Bartley (1939) have described other actions of strychnine, such as a diminution in accommodation and a prolongation of the period of latent addition, which, they believe, account for its central action. Such reactions to strychnine as these would, of course, tend to enhance any rôle the increase in amplitude of the oscillations in excitability might play in the induction of the central response to strychnine.

In so far as the foregoing considerations are valid they apply also to the enhancement of reflexes induced in the mammalian cord by cold, since while increasing the height and duration of the spike, cold increases the amplitude of the spontaneous oscillation; but cold raises the threshold more than does strychnine. Cold also diminishes accommodation.

Evidences of a Seasonal Variation in Amplitude of Oscillations. All of the determinations of the original series (Blair and Erlanger, 1933) were made during the second half of the month of May. As stated above, the average range of the oscillations in that series was about 2 per cent. In the case of the present series, the average of the first normal determinations of the preparations made during November to March, inclusive, is 3.2 per cent and of those made since then (beginning April 28th, but excluding the one of June 16th), 2.1 per cent. And several other determinations of the normal, made during June but not included in the table, have yielded percentages below 2. The determination of June 16th was made during a "cold" spell and gave a value of 3.2 per cent. This experience seems to indicate that "cold adaptation" increases the oscillation amplitude. The values for the *full* oscillations listed by Pecher (1939) range between 4 and 10 per cent and average 5.5 per cent; he gives neither temperatures nor dates.

GENERAL DISCUSSION. Three of the agents we have tested, namely, anode and cathode polarization and sodium citrate, are supposed to exert their effects on excitability through action on the "membrane". It is noteworthy that though all markedly affect electrical excitability, anode polarization lowering it and cathode polarization and citrate raising it, their action on oscillation amplitude in no case is striking if, indeed, they affect it at all. On the other hand, cold must affect all fiber mechanisms, and the fact that it enhances the oscillations while lowering excitability, considered in relation to the action of the above-mentioned agents, suggests that the oscillation response is not of the "membrane". The site of action of strychnine on the fiber still remains to be determined; but the fact that it lowers excitability while increasing the oscillations, may be taken to indicate that strychnine, also, produces the latter effect through action on something other than the hypothetical "membrane". But in view of its

nebulous state, the further development of this notion obviously is premature.

Previous work has disclosed evidences of subthreshold oscillations under certain imposed conditions. For instance, when frog's fibers are made to discharge repetitively through continuous superliminal cathode polarization there may be "gaps" in an otherwise regular series of responses. These gaps have durations which are about equal to two, or to three, or, rarely, even to four of the regular spike intervals, indicating that the gaps "result . . . from failures of an uninterrupted rhythmical oscillation in excitability to elicit a response" (Erlanger and Blair, 1936). And when the strength of a constant current is below the repetitive threshold, but above the rheobase, the make spike may be followed at the stimulated locus by a periodic variation in excitability decrementing through three recognizable oscillations (Erlanger and Blair, 1936). Monnier and Coppée (1939) have described a similar oscillation persisting after a brief subthreshold stimulus given to a nerve subjected to the influence of continuous superrheobasic cathodal polarization or of sodium citrate.

Arvanitaki (1939) has succeeded in recording local oscillatory variations in potential produced in giant fibers by cathode polarization of a locus treated with sodium citrate. And Brink and Bronk (1941) have produced similar oscillations in giant axons merely by removing the calcium and magnesium from topically applied sea water.

When these oscillations, however caused, develop under conditions that permit of the determination of their frequency it usually is found to be of the order of 200 to 300 per second. It remains to be determined whether the variations in excitability observed in the present investigation are periodic and if so what their rate may be. At the moment, therefore, it is not possible to assert that the oscillations in excitability and the rhythmical subconducted variations in potential are manifestations of one and the same phenomenon and therefore have the same frequency.

The faster rhythms exhibited by bits of frog's central nervous system may have a rate as high as 150 per second. This rhythm, and other central rhythms, have been attributed to "spontaneous activity of the cells" (Gerard and Young, 1937). The spontaneous oscillations of excitability we have studied are manifestations of nerve fibers. Is it possible that this fiber activity and this electrical activity of the central nervous system are fundamentally the same? If so we have here still another justification for regarding "axons as samples of nervous tissue" (Gasser, 1939).

The results we have obtained through the use of these agents fail to substantiate Pecher's inference that the oscillations are produced by molecular perturbations. Though cold would increase the mean free path of molecules, it is inconceivable that strychnine would have this effect.

SUMMARY

The amplitude of the spontaneous oscillations in excitability exhibited by single fibers in the phalangeal nerve of the frog is not changed by more than the error of the method, by

a. Anode polarization of a strength that raises the threshold as much as 70 per cent, or by

b. Cathode polarization that lowers the threshold 42 per cent, or by

c. Sodium citrate that lowers the threshold as much as 79 per cent.

d. The oscillation amplitude is definitely increased by cold which raises the threshold, but usually not more than 50 per cent.

e. The oscillation amplitude is increased as much as 394 per cent by a solution of strychnine which may not raise the threshold more than 12.3 per cent.

The possible relation of this effect of cold to the increase in excitability of the central nervous system that is produced by cold, and the possible relation of this action of strychnine on fibers to its central convulsant action are discussed.

The effect produced by strychnine on the oscillation amplitude renders untenable the view ascribing the oscillations to molecular perturbation (Pecher, 1939); and both the strychnine effects and the effect produced by cold suggest that the oscillations are not attributable to processes originating in the "membrane."

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THE EMPTYING TIME OF THE STOMACH OF OLD PEOPLE

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In a recent review Ivy (1) has noted the lack of quantitative information concerning important functions of the digestive system of the aged. Study of the normal physiologic functions of old people is basic to all phases of geriatrics. The present investigation was undertaken to determine what effect, if any, the aging process has on gastric emptying.

During the past few years the senior author and his colleagues have determined, under controlled conditions, the gastric emptying time of 59 vigorous, young men. These data have been of invaluable help in the present study, since they serve to establish a criterion for the emptying time of the stomach of young adults in their physical prime. The data obtained from the studies of gastric emptying of the aged group reported in this paper could be compared directly to those obtained from the young adults.

METHOD. Twelve men, the youngest of whom was 58 and the oldest 84, and whose average age was 70.8 years, were used for this study. Ten of the twelve subjects were indigents residing in the county infirmary; one was a college professor; one was a janitor.

At 7:30 a.m. they were given a high carbohydrate test meal similar to that used in previous studies (2). This meal consists of 15 grams of Quaker Farina and 350 cc. of water, boiled together and evaporated to 200 cc.; 50 grams of barium sulfate were added so that the position of the meal could be observed fluoroscopically. No food had been eaten since the preceding evening. The subjects were instructed to relax mentally and physically, but were allowed to walk around the laboratory if they so desired. The same methods were employed in all respects as for the estimations of gastric emptying time of young adults.

With the exception of 2 subjects, at least 3 determinations of gastric emptying time were made on each individual at exactly weekly intervals, to establish the mean for each subject. Time of emptying of the stomach as ascertained by fluoroscopic observation was determined to the nearest 10 minutes.

RESULTS. The results are expressed graphically in figure 1. The mean values for each of the aged subjects are superimposed upon a diagram of

distribution of the individual mean values for 59 young adults. The average length of time for the test meal to leave the stomach in the 12 old men was 1.94 hours, with extremes of 1.33 and 2.75 hours. The median value was 2.04 hours. The gastric emptying time of the 59 young adults averaged 2.08 hours, with extremes of 1.03 and 3.08 hours and a median value of 2.06 hours. It is apparent, without statistical analysis, that there is no significant difference between gastric emptying times of the 2 groups.

Discussion. Recently it has been pointed out (3) that gastro-intestinal symptoms are common in old age although gastro-intestinal disease is relatively uncommon. None of the subjects used for the studies reported in this paper, as far as could be determined, suffered from organic disease of the gastroenteric tract or complained of gastric disturbances at the times

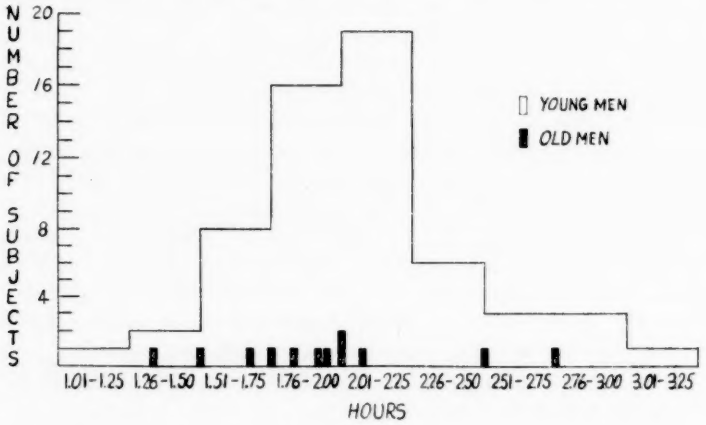

Fig. 1

the observations were made. They had normal appetite for food and regularly ate three meals each day. None suffered from cardiac embarrassment or other apparent causes of anoxic or stagnant anoxia, which could affect gastric emptying. All had sufficient cardiac reserve to perform light, physical tasks without distress. All but 2 coöperated well during the 4 weeks interval of study.

The subjects remained indoors during tests, since external environmental temperature may influence gastric emptying. The observations were made during December and January, while those on the 59 young adults were made during the cool months from October to May; thus was offered some degree of control of any possible seasonal variation in gastric emptying time.

Any greater activity of the younger group during the evening preceding the test meal, which is a factor difficult to control, presumably had little ef-

fect on mean values. If activity were greater, it should cause hunger, and Ivy and Fauley (5) have shown that hunger decreases gastric emptying time in experimental animals. The results indicate, however, that the younger group did not have a shorter gastric emptying time.

An interval of 7 days between tests is sufficient to rid the intestinal tract of all traces of barium sulfate. If much shorter periods were used, it is possible that a mass of barium sulfate in the lower intestinal tract could reflexly impede gastric emptying. Reflexes from the colon have been shown by Pearcy and Van Liere (6) to influence gastric motility.

Meyer and Necheles (3) have reported that no free acid was found in the fasting stomachs of 65 per cent of 29 patients over 60 years of age, and that salivary, gastric and pancreatic secretions were reduced in amount and content of enzymes, except for pancreatic amylase. An achlorhydric stomach may empty faster than the normal, but Ivy (1) has noted that this is not definitely proven. None of the 12 subjects was willing to submit to tests of gastric acidity, but even if it is granted that the majority were achlorhydric, the results still indicate that the gastric musculature of the aged is capable of exerting vigorous and effective peristalsis.

In the present small series, no correlation was observed between physical vigor and gastric emptying time. One of the least vigorous subjects had the most rapid emptying time.

Although there is no difference between gastric emptying times of old people and young adults, this should not be interpreted to mean that the aged should eat as much or even the same quality of food as is eaten by healthy, young adults. An assumption that gastric emptying time is a main criterion for eating habits is unwarranted, particularly in view of diminutions in the aged of secretions of the various digestive organs (3). Also, since many people past middle age lead sedentary lives, obesity results if eating habits are not suitably modified, and life expectancy may thereby be decreased.

SUMMARY AND CONCLUSIONS

The normal gastric emptying time of 12 men, the youngest of whom was 58, the oldest 84 and whose average age was 70.8 years was determined fluoroscopically. None of these subjects had demonstrable organic disease of the gastroenteric tract, and all were capable of performing light, physical tasks. The determinations of the gastric emptying time were made exactly at weekly intervals.

The criterion used for establishing normal gastric emptying time was the data secured from 59 vigorous young adults, who had been given the same type meal and subjected to exactly the same procedures as were the aged subjects.

The gastric emptying time of the 12 old men was 1.94 hours; the extremes

ranged from 1.33 to 2.75 hours. The gastric emptying time of the 59 young adults was 2.08 hours with extremes ranging from 1.03 to 3.08 hours.

It is concluded that gastric emptying is not influenced by senescence.

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OPTIMAL NaCl CONCENTRATION FOR ORAL SALINE DIURESIS[1]

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With the exception of the few workers who have ingested (0.9 per cent) isotonic NaCl and found it not to be diuretic, there have been few studies on oral NaCl solutions as diuretic fluids. Some physicians, not realizing that the sweat contains only 0.3 to 0.6 per cent of inorganic solids, have gone so far as to recommend 0.9 per cent NaCl solution as a drinking beverage for workers in hot atmospheres. Most clinicians have learned from edematous patients that one liter of 0.9 per cent NaCl supplies sufficient salt for the ordinary twenty-four hour requirements when no abnormal NaCl loss is occurring. It was thought that a careful study of the diuretic efficiency of a series of hypotonic concentrations of NaCl, as well as of the chlorides of calcium, magnesium, and potassium, might form an adequate basis for the future study of diuretics and at the same time furnish data on the all-important water and salt balance of the body. It is also known that while 0.9 per cent NaCl solution contains too much salt to be mobilized from the tissue spaces for diuretic purposes, at the opposite extreme the lack of salt in distilled water might inhibit diuresis when a larger dosage is used. Accordingly, the study of the diuretic effect of different concentrations within the range between these two extremes was first undertaken.

EXPERIMENTAL. All experiments were performed on female dogs which had been operated to produce bladder-extrophies (Dragstedt and Dragstedt, 1928). The animals were trained to stand quietly in stocks for the duration of each experiment, which lasted approximately three hours. Urine was collected at ten minute intervals from the funnels strapped under the fistulae. The urine volumes were measured and the chloride excretions were determined by a modified Volhard method (McLean and Selling, 1914). Water and food were withheld from the animals for five hours preceding each experiment to permit absorption of any recently ingested food or water. A control period of thirty minutes was allowed before the

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TABLE 1

Successive daily saline diuresis—100 cc./kgm.

		1st day	2nd day	3rd day
0.9% saline	Serum NaCl change	+71 mgm. %	+21 mgm. %	+66 mgm. %
	Urine peak	19 cc.	31 cc.	36 cc.
	Peak NaCl*	228 mgm.	337 mgm.	310 mgm.
0.5% saline	Serum NaCl change	−8 mgm. %	+22 mgm. %	+35 mgm. %
	Urine peak	43 cc.	55 cc.	54 cc.
	Peak NaCl	73 mgm.	81 mgm.	67 mgm.
0.2% saline	Serum NaCl change	+15 mgm. %	+15 mgm. %	+3 mgm. %
	Urine peak	50 cc.	57 cc.	56 cc.
	Peak NaCl	9 mgm.	42 mgm.	46 mgm.
Dist. H_2O	Serum NaCl change	−15 mgm. %	−15 mgm. %	−30 mgm. %
	Urine peak	40 cc.	45 cc.	48 cc.
	Peak NaCl	10 mgm.	15 mgm.	22 mgm.

* Expressed as mgm. NaCl/10 min./10 kgm. of dog. (See figs. 2 and 3.)

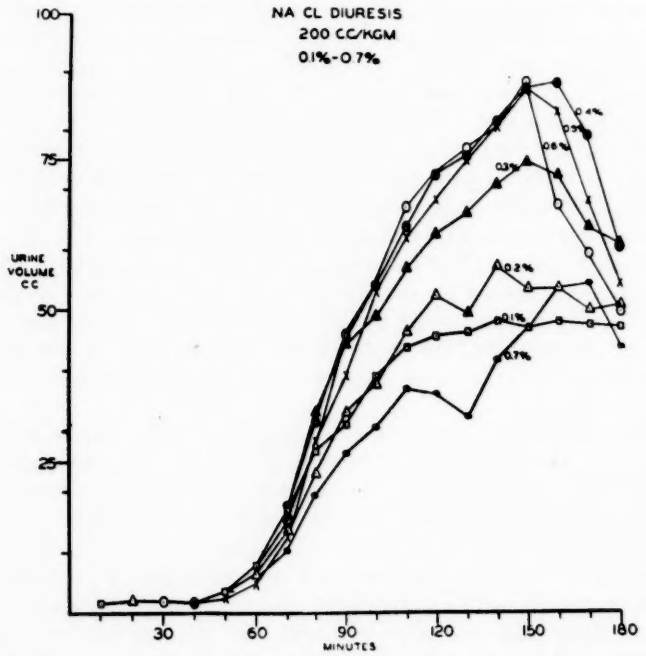

Fig. 1. The comparative maximum diuresis produced in the 10 kgm. dog by solutions of 0.1 per cent to 0.7 per cent NaCl when 200 cc./kgm. is given in 50 cc./kgm. doses at 30, 60, 90 and 120 minutes. The maximum diuresis occurs with doses of 0.4 per cent to 0.6 per cent, and this was found to obtain for doses of 100 to 300 cc./kgm. The diuretic response to the 0.7 per cent solution attains only the level produced by the more hypotonic solutions of 0.1 per cent and 0.2 per cent.

administration of any fluid. All fluids were administered by stomach tube at 37°C.

It was determined that a 50 cc./kgm. volume is well tolerated by the dog's stomach and that this quantity disappears from the stomach in

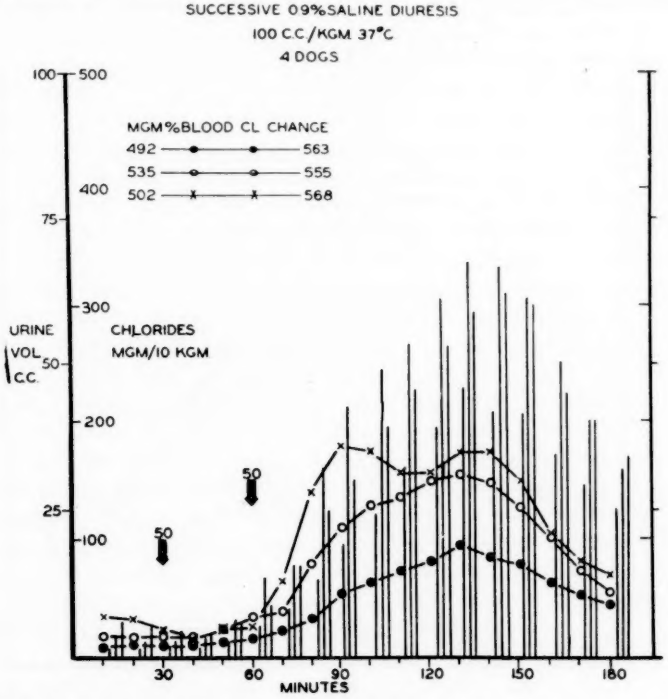

Fig. 2. Daily 0.9 per cent NaCl diuresis at 100 cc./kgm. The time of onset and peak of diuresis increased from day to day. The vertical lines on the inside of the ordinate measure the milligrams NaCl/10 min./10 kgm. The individual lines in each triad indicate respectively from left to right the height of chloride excretion at the corresponding time on the three successive days. The arrows indicate the time of administration of 50 cc./kgm. of the saline solution. The average blood chloride of the four dogs taken at the beginning and end of each experiment show a daily increase.

thirty minutes. Therefore, in these studies fluids were given in doses of 100 to 300 cc./kgm., divided into doses of 50 cc./kgm. administered at half-hour intervals. Four or five dogs were used in each experiment. Since the dogs used weighed approximately 10 kgm., all results were expressed on the basis of a theoretical 10 kgm. dog. The urine samples from all dogs in the group were pooled at each ten minute interval, and

a 5 cc. aliquot was taken for chloride estimation. Every thirty minutes the volume and chlorides of ten minute samples were determined for each individual dog to ascertain individual variation. Dogs which deviated from the average were replaced. A second series of experiments was performed to determine the effect of various concentrations of NaCl and also distilled water when administered three days in succession in doses of 100

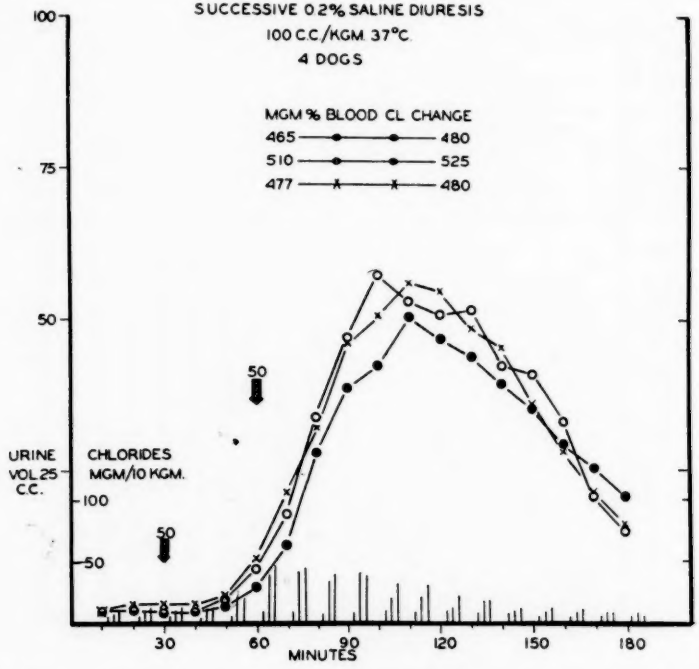

Fig. 3. Daily 0.2 per cent saline diuresis at 100 cc./kgm. More equal daily diuretic responses are obtained than with either 0.9 per cent or 0.5 per cent NaCl with respect to onset, peak and decline of diuresis. This may indicate that the disturbance in salt and water balance with this solution is minimum and easily compensated within 24 hours. No definite change in blood NaCl occurred.

cc./kgm. The blood chloride changes were followed daily with a sample obtained from each dog before the experiment and after the experimental diuresis. The samples were pooled and the chloride determination made in duplicate by the method of Koch. The summarized results are tabulated in table 1 and in figures 1 to 3.

Discussion. Oral administration of saline solutions ranging in concentration from distilled water to 0.9 per cent results in varying diuretic

responses, with the greatest diuresis occurring with solutions of 0.4 per cent to 0.6 per cent NaCl concentration. The limits of chloride excretion so far obtained in the normal 10 kgm. dog are 0.1 mgm./min. and 51 mgm./min. The difference between the diuretic response of the dog to 0.2 per cent and that to 0.5 per cent saline may represent the difference between sufficient chloride intake with 0.2 per cent saline and true chloride diuresis with 0.5 per cent NaCl. The oral administration of saline solutions for three days in succession results in the least average blood chloride change when 0.2 per cent NaCl is used. This concentration also furnishes adequate NaCl for the excretion of maximal quantities of fluid. Even with the extreme dilution of 0.1 and 0.2 per cent NaCl, a retention of over half of the NaCl occurred during the 3 hour diuretic period. The daily increase in urinary output is believed to indicate an increase in extracellular and tissue NaCl and, hence, an ability of the saturated tissues to absorb less NaCl and excrete more by way of the urine. This also indicates that even 0.5 per cent NaCl given orally is retained to such an extent that the dog is not in normal salt balance twenty-four hours later. Attempts were also made to substitute 0.5 per cent $NaHCO_3$ and 0.5 per cent NH_4Cl as a maximal diuretic fluid in order to study the effect of acid base balance on diuresis. These solutions caused marked vomiting and diarrhea and could not be given in doses greater than 50 cc./kgm.

In an attempt to verify these results in the human subject, three males ingested over a period of 90 minutes 50 cc./kgm. (4 liters) of 0.5 per cent NaCl solution. After an interval of one week the same amount of 0.25 per cent saline was ingested, and the next week distilled water was used. The 0.5 per cent saline experiment produced marked diarrhea in all three subjects. The 0.25 per cent saline was well tolerated and produced a diuresis almost comparable to the distilled water ingestion. The ingestion of 4 to 5 liters of distilled water over a period of one hour produced a headache in one subject and malaise in the other two subjects. Since the greatest diuresis accompanied the distilled water, it was thought that 50 cc./kgm. was not sufficient distilled water to exhaust the NaCl reserves of the body. The subjects noted a sustained diuretic effect from the 0.5 per cent saline, which carried over to the second and third day after the experiment.

With these results in mind, it is of interest to review the findings of Abel (1914), who in order to avoid "edema" in his vivi-diffusion experiments found it necessary to reduce the NaCl content to 0.55–0.6 per cent. Clark (1913) also found that the ordinary Ringer solution is improved if 0.3 to 0.4 per cent of the NaCl is omitted and the solution made isotonic with sucrose.

There are probably two underlying factors involved in the findings that 0.5 per cent NaCl is the most diuretic in dogs, while only 0.2 per cent is

needed for maintenance of salt balance. The absorption from the gastro-intestinal tract is more rapid when 0.5 per cent is used, but once this is absorbed the kidney cannot excrete more than 0.2 per cent NaCl in diuretic quantities. The vomiting which occurred with the lower concentrations of NaCl was probably due in part to the retention in the stomach of the dose of fluid previously administered.

SUMMARY

When bladder-extrophied dogs are given orally solutions of NaCl from 0.0 per cent to 0.9 per cent, it was found that 0.5 per cent NaCl produces the greatest diuresis; 0.2 per cent NaCl produces the least change in the NaCl balance, as shown by lack of cumulative action and minimal change in the serum chlorides. Human subjects cannot tolerate water containing more than 2 grams/liter of NaCl when large doses are given.

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THE DIURETIC EFFECT OF POTASSIUM, CALCIUM AND MAGNESIUM GIVEN ORALLY IN SALT SOLUTION

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It has been known since the pioneering experiments of Ringer (1882) on the perfusion of the frog heart that potassium, calcium, and magnesium each contribute to the automaticity of heart muscle. Aside from the clinical data which attest to the diuretic activity of these three ions in overdosage, little is known regarding their diuretic effect on the intact animal when used in a physiological range of concentration. Accordingly, diuretic experiments were planned in which these ions in physiological concentration would be added to the physiological, and most diuretic, concentration of NaCl.

TABLE 1

EXPERIMENT	DOGS	CC./KGM.	NaCl	K	Ca	Mg
			per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
I	8	200	0.45			
II	8	200	0.45	18		
III	8	200	0.45		10	
IV	8	200	0.45			3.5
V	8	200	0.45	18	10	
VI	8	200	0.45	18	10	3.5

Since the serum level of NaCl, 0.45 per cent, was found to be the most diuretic in previous experiments, a solution of this concentration was used as an appropriate medium in which to administer the chlorides of potassium, calcium and magnesium. Four trained, female, bladder-extrophied dogs were used in these studies. Experiments were performed at weekly intervals to allow the animals to return to a normal salt and water balance. Two experiments were performed at each saline concentration, making a total of eight dogs for averaging purposes. Solutions were administered by stomach tube in such quantities that each animal received a total of 200 cc./kgm. This was divided into four doses which were given

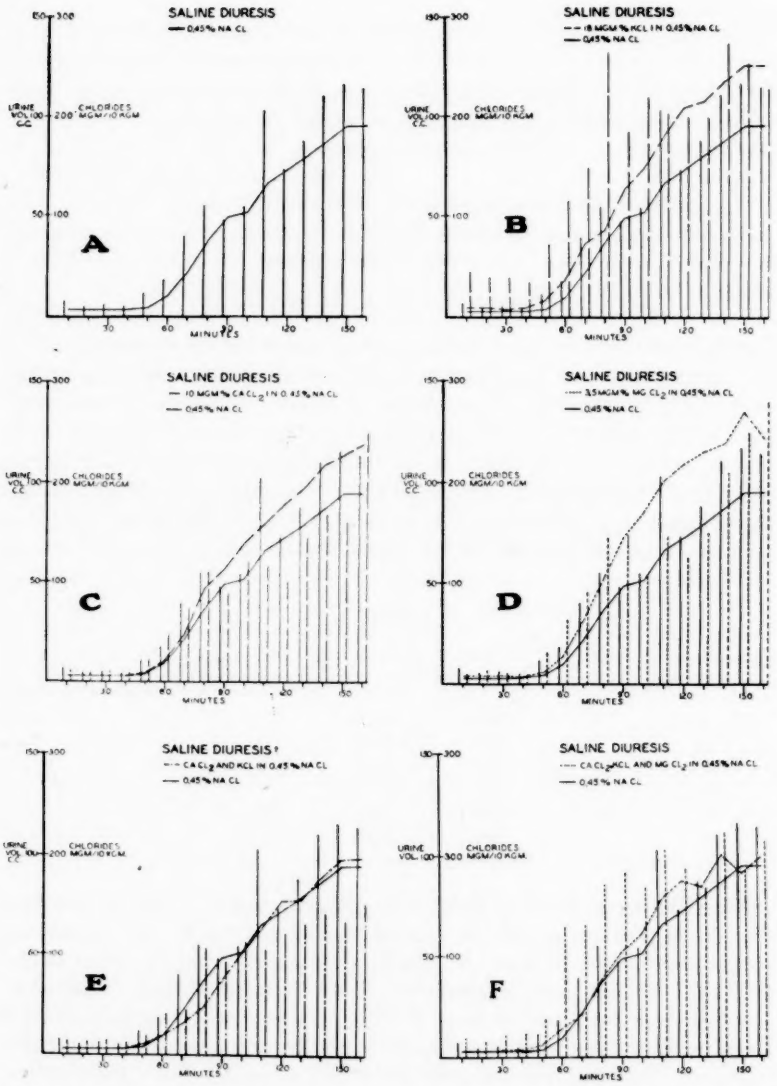

Fig. 1. Data are expressed on a basis of a 10 kgm. dog when eight dogs received 200 cc./kgm. orally in doses of 50 cc./kgm. at the 30, 60, 90 and 120 minute intervals. The milligrams chloride excretion, expressed as NaCl, is represented by the vertical lines.

at half-hour intervals after an initial thirty minute control period. Table 1 represents a protocol of the experiments conducted.

RESULTS. The graphs of figure 1 summarize the experimental data. After determining the normal response to 0.45 per cent NaCl, KCl was added in an amount to provide 18 mgm. per cent of available K ions in the diuretic fluid. For purposes of comparison this curve is superimposed on the control NaCl graph. The onset of diuresis is earlier and a higher peak is attained. The chloride excretion, as represented by the broken vertical lines, appreciably exceeds that of the 0.45 per cent NaCl alone.

When $CaCl_2$ was added (Ca^{++} = 10 mgm. per cent), the water diuresis exceeded that of the control curve. The chloride excretion in the 90 to 150 minute period was less than that of the control 0.45 per cent NaCl experiments.

$MgCl_2$, when added in sufficient quantity to provide 3.5 mgm. per cent of available Mg ions, resulted in a marked increase in water diuresis over the entire period of the experiment. The NaCl output, except for the 50 to 90 minute period, equaled that of the 0.45 per cent NaCl.

The combination of Ca (10 mgm. per cent) with K (18 mgm. per cent) resulted in a loss of the normal diuretic effect of these ions. A definite retention of chloride started at 90 minutes and obtained for the duration of the experiment.

The addition of Mg (3.5 mgm. per cent) in the presence of Ca (10 mgm. per cent) and K (18 mgm. per cent) decreased the diuretic effect of the Mg, Ca and K ions. A greater chloride excretion occurs with this combination than with the 0.45 per cent NaCl alone.

DISCUSSION. When K, Ca, and Mg chlorides are given singly in a diuretic fluid in the same concentrations in which they appear in the blood stream, the resulting diuresis is at all times greater than that seen with 0.45 per cent NaCl alone. In the case of a balanced solution containing Ca and K in 0.45 per cent NaCl, the data point to a retention of chloride ions and a total reversal or loss of the diuretic effect of these two ions. The diuretic effect of $MgCl_2$ is also decreased by administration in a balanced Ca-K-Na solution. The explanation of these phenomena must await further studies checking blood volume and tissue water balance. The lack of water diuresis might be accounted for by increased urinary concentration. The decrease in chloride excretion, however, would indicate an actual retention of these ions. This antagonism between Ca and K has been confirmed in water balance studies in rats.[1] These results are at variance with those observed with the heart-lung-kidney preparation (Eichholtz and Starling, 1925). When K or Ca chlorides were given

[1] To be published.

separately, no effects were noted in the excretion by the isolated kidney. If these two salts were given together, however, there was an increase in chloride excretion and water output. The concentrations used in those experiments, however, exceeded the normal physiological range.

SUMMARY

By adding each of the serum cations to an oral 0.45 per cent NaCl solution it is possible to ascertain the diuretic effect of each ion. The addition of Ca is the least diuretic, while Mg addition results in the greatest diuresis. K is intermediate. The combination of Ca and K prevents the diuretic action of each ion and results in a greater retention of chlorides. Mg does not completely enter into this antagonism, although the diuretic effect of this ion is greatly diminished.

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THE ENZYMATIC INACTIVATION OF CHOLECYSTOKININ BY BLOOD SERUM

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We have recently demonstrated (1) that blood serum contains "secretinase," the presumed action of which is to remove active secretin from the circulation and permit pancreatic secretion to subside. We believe this enzyme to be an important factor in the regulation of the external secretion of the pancreas.

In the normal digestive process the hormonal stimulus for pancreatic secretion is accompanied by an emptying of the gall bladder which is due to contraction of its musculature mediated by the hormone cholecystokinin (2). The characteristic gall-bladder contraction in response to cholecystokinin stimulation is abrupt and reaches its height in one or two minutes; this is followed by slow relaxation. The extent of contraction and the interval required for relaxation depend to a large extent on the quantity of cholecystokinin administered. When an extract of intestinal mucosa containing both secretin and cholecystokinin is injected and the responses of the pancreas and gall bladder are observed, the parallelism is striking with respect to onset, magnitude and duration of action of the two hormones. A similar parallelism obtains in the case of their endogenous absorption in the course of the normal physiologic response to a meal. In general on intravenous administration the cholecystokinin effect is the more protracted.

Since secretin and cholecystokinin enter the circulation from the same source (the upper intestinal mucosa) and appear to be closely related chemically, and since their separate effects coincide to some extent, a similar or identical agency in clearing them from the circulation appeared to us to be probable. The presence of such a mechanism was tested in the experiments described below.

EXPERIMENTAL. 1. *Materials.* The same S_1 preparation employed by us in our previous work served as the cholecystokinetic agent. This material, which contains one unit of secretin in 0.25 mgm., causes a 1-cm. rise in intra-gall-bladder pressure in most dogs in a 0.5 mgm. dose. In the present work injections of 2 mgm. were made in order to secure pronounced reac-

tions and accentuate any differentiations in effectiveness encountered in the course of treatment by serum. Normal dog serum was used throughout these studies.

2. *Methods.* Large dogs (15 kilos or over) were anesthetized with sodium pentobarbital and the abdomen was opened by a midline incision. The cystic duct was clamped and a trocar inserted in the dome of the gall bladder and connected to a Becker tambour to provide for a recording of gall bladder motility (2). Provision for recording pancreatic secretion and carotid blood pressure was made as in our previous experiments. The response of the animals to a control injection of 2 mgm. of S_1 was established, and mixtures of S_1 and serum were injected under the following conditions: (a) *Constant:* Volume, pH, and temperature of serum.

TABLE 1

Progressive disappearance of cholecystokinin from solutions of 2 mgm. S_1 in 10 cc. dog serum at varying incubation times (37°C.)

DOG NO.	GALL-BLADDER RESPONSE													
	Rise	Duration	Rise	Duration	Rise	Duration	Rise	Duration	Rise	Duration	Rise	Duration	Rise	Duration
	cm.	*min.*												
1	3.7	12	3.5	12	3.3	8	3.0	7	0	0			4.0	15
2			2.0	9	1.8	8	1.5	6	1.0	4			3.0	17
3					4.0	18	3.0	15	2.0	8			3.5	14
4	5.3	13	4.1	11			1.7	16	0.7	4	0	0	5.0	12
5	6.7	15	4.5	12	4.0	9	2.0	8	1.8	6	0	0	6.1	14
6	4.8	9	4.6	8	3.3	7	2.5	7	2.3	7	0	0	5.0	10
	Incubation time (hours)													
	0		½		1		2		3		5		Control	

Varied: Time of incubation. (b) *Constant:* Volume and pH of serum and time of incubation. *Varied:* Temperature of incubation. (c) *Constant:* Time and temperature of incubation and pH of serum. *Varied:* Volume of serum. (d) *Constant:* Time and temperature of incubation and volume of serum. *Varied:* pH of serum.

The degree of excursion of the Becker tambour was standardized with reference to the observed height of the visible column of bile, so that the records obtained could be recorded in terms of changes in intra-gall-bladder pressure in centimeters of bile.

RESULTS. The gall-bladder response to the intravenous injection of S_1-serum mixtures was altered in a manner analogous to the diminution previously noted in the response of the pancreas to the serum-treated material. The degree of inactivation of cholecystokinin was found to vary directly with the length of time of incubation, and was usually complete in

5 hours when 2 mgm. of S_1 was treated with 10 cc. of serum. The degree and duration of gall-bladder response in this series of animals is listed in table 1, and an illustrative record of one experiment is reproduced in figure 1. The inactivation was found to proceed slowly at low temperatures, optimally at body temperature, and was entirely eliminated by heating the serum to over 60°C. prior to incubation (table 2). The degree of inactivation for a given time and temperature was a function of the amount of

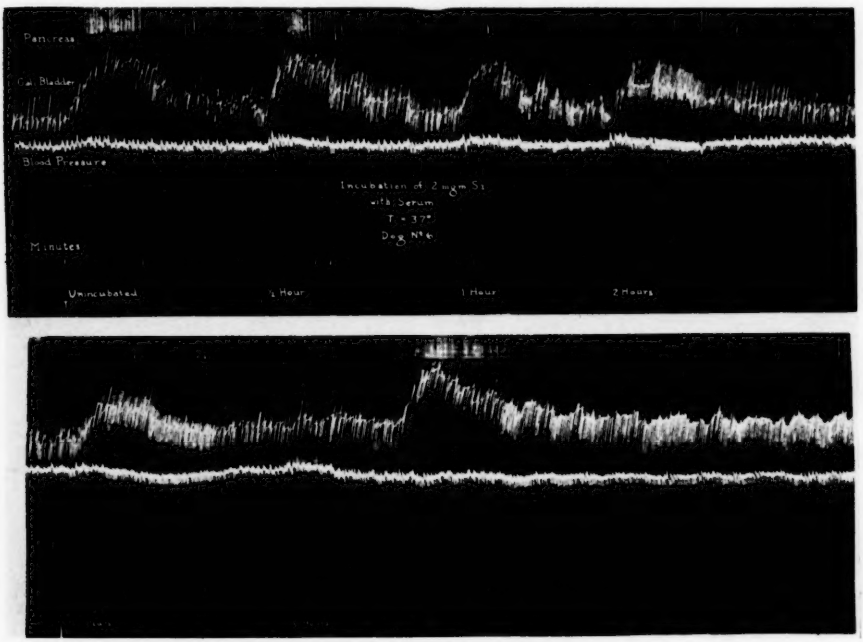

Fig. 1. Progressive inactivation of the secretin and cholecystokinin in 2 mgm. of S_1, following incubation with 10 cc. of normal dog serum for varying lengths of time. The secretin is entirely inactivated after 3½ hours, and the cholecystokinin after 5 hours.

serum used (table 3), and the inactivating agency was ineffective when the acidity or alkalinity was caused to deviate markedly from the normal pH of the blood (table 4).

It was not possible to evaluate alterations in gall-bladder response with the same degree of accuracy as in the case of the pancreas. The nature of the recording apparatus is such that only sizable differences in response are significant; and we were restricted to a small number of injections in any given animal, since it is known that frequently repeated stimulation of

the gall bladder by cholecystokinin will eventually throw the viscus into a state of persistent contracture which renders the animal useless for assay purposes.

TABLE 2

Effect of temperature of incubation upon gall-bladder response to 2 mgm. S_1 incubated with 10 cc. of serum

DOG NO.	GALL-BLADDER RESPONSE									
	Rise	Duration	Rise	Duration	Rise	Duration	Rise	Duration	Rise	Duration
	cm.	*min.*								
7	1.5	14	0.5	7	0.2	3			1.4	14
8	2.3	15	1.7	12	1.2	9			2.5	18
9	4.0	25	3.3	18	2.0	15			3.3	25
10	2.0	8	0.7	6	0	0				
11	1.0	6	0.8	6	0.5	4			1.0	6
12	1.2	9	0.9	9	0.6	4			1.2	9
13					0.5	3	2.5	18	2.2	18
14					0	0	1.8	15	1.2	13
	Temperature, degrees C.									
	10		22		37		60		Control	

TABLE 3

Effect of varying quantities of serum on gall-bladder response when incubated with 2 mgm. S_1 at 37°C. for 4 hours

DOG NO.	GALL-BLADDER RESPONSE											
	Rise	Duration	Rise	Duration	Rise	Duration	Rise	Duration	Rise	Duration	Rise	Duration
	cm.	*min.*										
15	2.2	13	2.5	9	1.5	6	0.5	2	0	0	2.5	11
16	2.5	14	1.7	8	0.8	5	0.6	4	0	0		
17	1.2	18			0.5	9	0.1	1	0	0	0.9	?
18	2.0	15			1.0	7	0.8	5	0.7	3	2.0	15
19	1.2	12	0.5	3			0	0			1.5	13
20	3.0	13	2.5	10	1.8	5	1.0	2	0	0	4.0	15
21	1.2	12	0.3	3	0	0	0	0			1.5	17
	Volume of serum (cc.)											
	1		2		5		10		20		Control	

Discussion. It is apparent from the data submitted that inactivation or destruction of cholecystokinin is brought about when this hormone is incubated with blood serum at the physiologic normal temperature and reaction. The process of removal of active cholecystokinin from such a

mixture is dependent on the time of incubation and the amount of serum used. It proceeds most rapidly at body temperature and is prevented by heating to 60°C. or by adjusting the reaction to an acidity or alkalinity deviating markedly from the normal (2 pH units or more). It follows that the mechanism whereby active cholecystokinin is caused to disappear from

TABLE 4

Effect on gall-bladder response to 2 mgm. S_1 incubated with 10 cc. of serum for 4 hours at normal, acid, and alkaline pH

DOG NO.	SERUM pH	GALL BLADDER	
		Rise	Duration
		cm.	*min.*
22	4.6	1.7	13
	7.0	0.2	3
	10.4	1.2	11
	Control	1.7	14
23	2.0	1.0	8
	7.0	0	0
	11.0	1.0	8
24	2.0	1.4	8
	7.0	0.7	5
	11.0	0.7	7
25	Control	2.2	12
	9.4	2.0	12
	7.5	1.2	6
	3.4	1.2	7
	Control	2.0	?
26	3.4	1.5	12
	7.5	0.3	3
	9.4	1.2	13
27	3.5	2.5	11
	7.5	1.0	5
	10.2	2.7	18
	Control	2.5	12

the incubation mixtures is an enzymic one, since the usual criteria for the demonstration of enzyme activity have been fulfilled by the experiments outlined above.

It remains to be established whether the same enzyme is involved in the inactivation of both secretin and cholecystokinin. It is quite conceivable that the two hormones are structurally similar chemically; witness their

close association *in vivo* and in processes for preparing concentrates. Thus it is entirely admissible that a single enzyme present in the blood destroys or alters a certain chemical group or atomic linkage essential for their separate activities. However, until the structures of the two substances are known, one can merely speculate regarding this possibility. It is certain that they are not identical; if additional proof of this fact were necessary, the present experiments supply it. Each injection of 2 mgm. of S_1 contained 8 units of secretin and 4 units of cholecystokinin; thus if a single substance were being destroyed by the blood serum, the cholecystokinetic effect would obviously have completely disappeared before the secretin potency had been lost. Exactly the reverse was actually obtained, as is illustrated in figure 1. It follows that the enzymic inactivation of secretin proceeds with considerably more rapidity than that of cholecystokinin.

SUMMARY AND CONCLUSIONS

A concentrate containing secretin and cholecystokinin has been incubated with normal dog serum and inactivation of the cholecystokinin has been demonstrated.

Such inactivation is progressive with time of incubation and is most rapid at 37°C. The agency involved is heat-labile and is effective only at a reaction approximating the pH of the blood. Thus the mechanism whereby inactivation occurs is an enzymic one.

The enzyme involved may or may not be secretinase. The inactivation of cholecystokinin proceeds considerably more slowly than does that of secretin.

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RED CELL COUNTS, PERCENTAGE VOLUME, AND THE OPACITY OF SUSPENSIONS[1]

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Several methods have been suggested as a means of avoiding direct red cell counting and hematocrit determinations, the former because it is tedious, and the latter because of the doubt which exists as to the right rate and time of spinning.[2] One method is described by Ponder, Dubin and Gordon (1934), and consists in finding the red cell count by dividing the percentage volume found by hematocrit by the mean cell volume in μ^3 found by diffraction. Another is that originally due to Oliver (1895–96) and developed by Holker (1921); the red cell count is determined from the opacity of a suspension, measured in various ways. Shohl (1940) has used the same principle for finding the percentage volume, the opacity of a suspension of red cells of the sample of blood being measured with the Evelyn photometer, and the percentage volume read off from a previously prepared linear calibration curve.

I re-examined the opacimeter method in 1934 (Ponder, 1934), and was unable to find the precision which Holker claimed for it, for the opacity of a red cell suspension turned out to be determined not only by the number of cells, but also by their size, their shape, and the refractive difference between them and the surrounding medium, as well as by other factors which are obscure. In general, the discrepancy between the results obtained by opacimetry and by direct counting were found to be sometimes as great as ± 10 per cent.

In these experiments, however, I used a Zeiss Stufenphotometer with a large angular aperture, and so both scattered light and directly transmitted light were measured together. The importance of the angular aperture has since been emphasized by Mestre (1935), and so better results might be obtained with a photometer of smaller aperture such as the Klett-

[1] This investigation was carried out by the aid of a grant from the Simon Baruch Foundation.

[2] For a description of the difficulties which arise in connection with the hematocrit method, see Ponder (1940) and the papers therein referred to. It should be again emphasised, however, that the value of the method depends on whether it is used for the determination of relative, or of absolute, volumes.

Summerson photometer.[3] I also used a filter with a maximum transmission at 5300 A, but Shohl has since pointed out, on the basis of Drabkin and Singer's investigations (1939), that a red filter with a maximum transmission at 6600 A ought to be better, as it minimizes the effect of variations in hemoglobin content. Lastly, my investigation was directed more to seeking the sources of error of the method than to seeing how it would work out in practice, which is the purpose of the present series of determinations.

METHODS. The samples of venous blood (about 5 ml.) were received into small bottles containing heparin. Oxalate should not be used, because of its effect on the cell volume and on osmotic properties (Ponder, 1940). The cases from which the blood samples were taken were selected so as to exclude the blood dyscrasias, and those showing low red cell counts were cases of either secondary anemia or hemorrhage.

The opacity measurements were made with a Klett-Summerson photometer, using *a*, the red filter 66 with a transmission of from 6400 to 7000 A, and *b*, the green filter 53 with a transmission of from 5000 to 5700 A. The suspension was prepared by adding 20 mm.^{3} of the heparinized blood sample to 10 ml. of 1 per cent NaCl or to 10 ml. of the citrate-formol solution described by Shohl (3.0 per cent sodium citrate to which is added 1 ml. of formalin for each liter). Readings of the photometer were made as soon as possible after adding the blood to the NaCl or citrate-formol and mixing, 30 seconds after mixing, 1 minute after mixing, and 2 minutes after. During this period the opacity almost always falls in NaCl suspensions, and invariably rises in citrate-formol, and at the end of 2 minutes a steady state is reached. To get information about stirring effects, the tubes were inverted at the end of 2 minutes, or their contents stirred with a small air jet. This usually results in an increase in the opacity within 15 seconds after the stirring, and a return to the original steady value within a minute.

The red cell counts were made on the samples of heparinized blood in the usual way, 5 squares being counted on each side of a double chambered Levy-Hauser counting chamber, and checked by a second pair of counts in cases where the deviation of the first pair exceeded $2\sqrt{n}/n$.[4]

[3] Shohl used the Evelyn photometer, which is of small aperature.

[4] While the theoretical standard error for the distribution of n cells is $\sqrt{n}/n$, as shown by "Student" in 1906, deviations of more than twice the standard error occur in practice with an unexpectedly high frequency. It is also noticeable that the error does not diminish as rapidly as it should when larger numbers of cells are counted, i.e., it is somewhat like the standard error of the polynuclear count (Ponder, Saslow and Schweizer, 1931). Attention should be called to the fact that there are considerable errors connected with the process of sampling, for blood in a vial, even if well stirred, is not altogether homogeneous; this is probably due to the cells being carried, en masse, in shoals by currents in the fluid, and is what gives rise, essentially, to the stirring errors described in this paper. In practice, there must be added errors in-

The hematocrit tubes were 100 mm. long, about 1 mm. in bore, and made of heavy walled glass. They were filled by means of capillary pipettes, and spun at 3000 r.p.m. for 15 minutes; this may not be an ideal speed and time, but results in the attainment of constant volume, and is satisfactory, in my experience, *for comparative purposes*. In measuring the length of the column of packed cells, the buffy coat was not included; at the same time, however, the length of the white cell column was measured, and a white cell count was done in the usual way. Hemoglobin was also determined on each sample.[5]

RESULTS. To express the relation between any pair of measurements (e.g., red cell count and opacity as measured with the red filter after 2 minutes, the cells being in citrate-formol), the figures for the count and for the opacity were plotted against each other, and a best line passing through zero was drawn through them by the method of least squares. For each opacity reading, the difference between the corresponding observed count and the count derived from the linear relation was obtained; the standard deviation σ of the deviations was then found in the usual way. This is better than calculating a coefficient of correlation between the two variables, because it gives a direct measure of the degree of scatter of the discrepancies and of the frequency with which a discrepancy of any given magnitude may be expected to occur. The values of σ for different pairs of measurements are shown in table 1. The magnitude and distribution of the stirring effects are shown in table 2, each stirring effect being expressed as a percentage of the photometer reading.

DISCUSSION. As regards the correspondence between red cell counts and opacity measurements, the most satisfactory agreement is found when

troduced by variations in shaking in the hemacytometer pipettes; we use a standard shaking, by hand, of 5 minutes, but a shaking machine is to be preferred.

[5] While the number of determinations made in the course of this investigation is not large enough for any very detailed statistical analysis, I have not been able to find any relation between the number of white cells present and the magnitude of the discrepancy between the observed count (or hematocrit reading) and the value given by the linear relation determined from the mass of the data. Shohl has pointed out that caution ought to be exercised in applying the opacity method to samples in which the number of white cells is greatly increased, and in this investigation I have rejected all observations for samples in which the total white cell count was less than 5,000 per mm^3. or more than 10,000 per mm^3. This means that the buffy coat did not exceed about 1 mm. in the hematocrit tube, and it is no doubt because of the restriction placed on the number of white cells allowable that no indication of their effect on the opacity appears in the figures. In some cases in which the white cell count was in the neighborhood of 20,000 to 40,000, the results obtained from the opacity measurements agreed very badly with those of red cell counting, as might be expected. Curiously enough, variations in the hemoglobin content have not been found to affect the discrepancy between opacimetry and red cell counting or hematocrit determinations, even when the green filter was used.

the cells are suspended in citrate-formol and when the opacity measurements are made with a red filter (maximum transmission 6600 A) and 2 minutes after adding the cells to the suspension medium.[6] Even under these circumstances, however, the standard deviation of the discrepancies between the count as made directly and the count as calculated from the opacity amounts to $\pm 0.545 \times 10^6$, i.e., about two-thirds of the discrepancies are less than this, but about one-third of them is greater.

At this point the question must arise as to the extent to which the discrepancies are contributed to by the red cell counts themselves being in error. The standard error which attaches itself to a red cell count by reason of the imperfection of the distribution of the cells on the squares of the chamber is $\sqrt{n}/n$ in theory ("Student", 1906), and somewhat greater

TABLE 1

Red cell count and opacity	Green	1st setting	NaCl	$\pm 0.511 \times 10^6$
	Red	1st setting	NaCl	$\pm 0.467 \times 10^6$
	Red	2nd setting	NaCl	$\pm 0.570 \times 10^6$
	Red	2nd setting	Citrate-formol	$\pm 0.545 \times 10^6$
Hematocrit and opacity	Red	1st setting	NaCl	± 3.11 units
	Red	2nd setting	Citrate-formol	± 2.70 units

TABLE 2

Distribution of stirring effects, per cent

	NEGATIVE	0-2	2-4	4-6	6-8	8-10	10+
NaCl	2	10	15	15	4	2	2
Citrate-formol	0	37	13	0	0	0	0

in practice (Ponder, Saslow and Schweizer, 1931). The average red cell count in the series for the red filter and citrate-formol was 4,270,000, and with 2 sides of the chamber counted, the number of cells seen, n, would be about 800; this gives a theoretical error of ± 3.5 per cent. The standard error of the discrepancy between the count obtained directly and the count calculated from opacity, however, is $\pm 0.545/4.27$, or ± 12.7 per cent, and so it is unlikely that the error of direct counting goes more than about halfway towards accounting for the standard error of the discrepancy. Errors

[6] Numerically the value of σ for the 1st setting with either the red or the green filter and a NaCl suspension medium is smaller, but not significantly so, and the stirring variations are more pronounced (table 2). All the stirring effects described here are quite different from the "spontaneous changes in light transmission" described by Kesten and Zucker (1928); the latter are observed after 2 to 3 hours, and are apparently due to the cells assuming the spherical form. They result in an increase in the opacity.

due to diluting and to obtaining a good sample of blood are common to both direct counting and opacimetry, and so cannot be held responsible.

Remarks of a similar kind apply to the relation between opacity and percentage volume as found by the hematocrit. Citrate-formol is again the preferable medium, and the red filter preferable to the green one, but the standard error of the discrepancies amounts to ± 2.7 units of volume. The hematocrit method may not be a very reliable one, but when used for relative purposes its standard error is certainly less than this.

There is every reason, indeed, to believe that the opacity of a red cell suspension is a function of several variables other than number and size (e.g., shape, refractive index differences, etc.), and perhaps the clearest evidence of this is that the correlation coefficient between the opacity as measured in citrate-formol and the opacity as measured in NaCl is only 0.9 ± 0.03, i.e., the two sets of measurements are not measuring quite the same thing, and there exist factors which do not influence them both identically. As Shohl points out, one should not lose sight of the fact that a disagreement between the results of red cell counting or the hematocrit on one hand, and of opacimetry on the other, does not mean that the former methods are necessarily right and the latter wrong, for the methods measure different physical properties of the blood and therefore all the results may be valid; the discrepancies, indeed, may themselves be significant, and may be correlated with as yet unidentified differences in the state of the cells. Further investigation is needed along these lines. The question nevertheless remains: Can the opacimeter method, in its present form, be used to replace red cell counts and hematocrit determinations for routine clinical purposes? Because of the size of the standard errors, I think that the answer must be no, for there is about 1 chance in 10 that opacimetry will give a difference of more than $\pm 500{,}000$ between two red cell counts when none exists. Direct red cell counting has always the advantage that any uncertainty can be removed by repeating the count so as to diminish the standard error, which can theoretically be reduced indefinitely by increasing n. For this reason it must remain the standard method.

The results do not throw much light on the nature of the stirring effects in suspensions, but several points emerge quite clearly. 1. The opacity of suspensions of red cells in citrate-formol is always greater than that of cells in NaCl, in part at least because the cells swell in citrate-formol (Shohl, 1940, Ponder, 1940). Whether the swelling is great enough to account for the opacity difference (opacity in citrate-formol = 1.48 times opacity in NaCl, on the average) is not clear. 2. With a very few exceptions, the stirring effects are always such as produce a greater opacity, and they are larger and more variable in NaCl than in citrate-formol. Their effect is just the opposite from the stirring effects met with in con-

ductivity work, where stirring produces a small decrease in resistance and capacity, or an increased conductivity (Fricke and Morse, 1925). 3. The effects on opacity and on conductivity are much greater than can be accounted for by changes in the orientation of individual cells. If we have a suspension of red cells in the path of a parallel beam of light or between two parallel electrodes, the opacity and resistance will depend, in a complex manner, on the effective area or "target area" which the cells present in in the direction of the light or of the current flow. To take extreme instances, all the cells might be oriented edge-on or all oriented face-on, and in the former case the opacity and resistance would be expected to be less than in the latter since the target area is smaller. In any real case the target area S will be somewhere between the two extremes. If S is the maximum target area of the single discoidal cell of radius r ($s = \pi r^2$), it can be shown (Cox, 1941) that $S = Ns/2$ and that its fractional mean square deviation is $1/3N$, where N is a number of cells oriented at random. This deviation is far too small to account for the effects of stirring on the basis of chance changes in the orientation of single red cells. We have rather to suppose that there is orientation of masses of cells on a large scale, the orienting forces being presumably currents in the suspension fluid (cf. Gorin and Velick, 1940). In this connection it may be remarked that in a suspension at rest the gravitational forces are not great enough to orient the cells in the position of greatest hydrodynamic stability, i.e., on the flat; stirring accordingly produces a change from a state of randomness to one of orientation, and not *vice versa*, as is often implied.

SUMMARY

Although the number of red cells and the percentage volume occupied by them can be found approximately by measuring the opacity of a suspension, the fact that the opacity is a function of variables other than number and size leads to results which often are in poor agreement with the results of direct counting and of hematocrit determinations. The standard error of the discrepancies between the count obtained by direct counting and that calculated from opacimetry is about $\pm 500{,}000$, and that of the discrepancies in percentage volume about ± 2.7 units of volume.

A solution of isotonic sodium citrate with 0.1 per cent formol added is preferable to isotonic NaCl as a suspension fluid for opacity measurements, but only because the variations in light transmission which accompany stirring are greatly lessened. These stirring effects seem to be due to the orientation of masses of cells by currents in the suspension medium.

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SOME EFFECTS OF COBALT AND LIVER SUBSTANCE ON BLOOD BUILDING IN DOGS[1]

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Polycythemia was first produced in rats by cobalt feeding in 1929 by Waltner and Waltner (1). This observation has been confirmed with rats and extended to dogs, pigs, rabbits, guinea pigs, mice, frogs and chickens. Thus, cobalt seems to possess the general biologic property of stimulating erythrogenesis when fed in physiologically massive doses.

The etiology of polycythemia is little understood. Although cobalt feeding, lowered oxygen tension, or extended regular muscular exercise, all stimulate the development of polycythemia, their exact modes of action are not known. Orten (2) suggested that cobalt causes vasodilatation, thus producing anoxemia, which constitutes a primary hematopoietic stimulus. Mascherpa (3), who first reported cobalt polycythemia in dogs, observed hyperplasia of the bone marrow and considered the action of cobalt to be directly on the erythropoietic centers. Kleinberg, Gordon and Charipper (4) produced anemia in rabbits by bleeding and by benzol injection, and induced rapid erythropoiesis by injection of cobalt. They concluded that cobalt stimulates the formation of erythrogenic precursors in the bone marrow.

Recently Davis (5) and Brewer (6) have contested the fact of whether or not a true polycythemia is obtained uniformly and maintained consistently in dogs fed high levels of cobalt. Brewer (6) subjected his own and Davis' data to statistical analysis and concluded that cobalt feeding actually caused a depression in blood values in some cases, although in general it caused a slight increase.

A satisfactory theory for the action of cobalt, or, for that matter, any

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A preliminary report of this investigation was presented at the Toronto Meeting of the American Society of Biological Chemistry, April, 1939 (Frost, D. V. and C. A. Elvehjem, J. Biol. Chem. **128:** xxi, 1939).

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causative factor of polycythemia is difficult to formulate with the facts at hand. Most reports of experimental work dealing with the condition fail to take account of the variable effect diet may play and it seems quite probable that this will need to be more fully understood and placed under experimental control before the picture of the physiological control of hematopoiesis can be assessed. In any condition, however, the possibility for uncontrolled variation is certainly large.

In studies extending over a period of five years in this laboratory the adequacy of milk diets with added iron, copper and manganese (7, 8) to support hematopoiesis in dogs has been demonstrated. Recently (9) the question of the need for cobalt for rapid blood formation was studied using this diet. Although some evidence indicated a stimulating effect of small amounts of cobalt on the rate of hematopoiesis, definite conclusions could not be drawn as to its essentiality. The adequacy of milk with added iron, copper and manganese was perhaps best shown by experiments (10) designed to compare the effect of iron and copper versus whole liver substance to support blood building. Copper and iron appeared to be about equal to liver in this capacity in these experiments. Thus there appeared to be no evidence for the existence in liver of special hematopoietic factors not supplied in adequate amount by milk. That liver does contain factor(s) qualitatively or quantitatively different from those in milk which can have a very profound effect on the hematopoietic function was discovered quite unexpectedly.

During the course of experiments (9) to determine the need for cobalt in dogs on milk diets we produced anemia in several adult dogs by phlebotomy. One of the dogs was fed a high level of cobalt about three weeks after severe anemia had been produced. The cobalt feeding had an apparent adverse effect on the appetite and general condition of the dog, but this was not surprising. However, the failure of iron and copper additions to initiate hematopoiesis after the cobalt feeding was indeed surprising, particularly in view of the fact that littermates which had been phlebotomized at the same time showed typical excellent responses to copper and iron alone. The continued failure of the dog on the cobalt, iron and copper regimen suggested the idea that cobalt feeding under these conditions might create an unnaturally large demand for certain hematopoietic precursors. The most likely material to test for such precursors was whole liver substance, and the response of the dog to liver feeding, despite continuation of the other therapy, was profound both as to the effect on general health and rate of hematopoiesis. The above experiment and further experiments of this type are described more completely below.

The finding that liver overcomes an apparent inhibition to hematopoiesis induced by cobalt stimulated our interest in the recent work of Davis who had reported results with cobalt and liver feeding which appeared in almost

direct antithesis to our results. Davis (5, 11) reported a stimulation of blood formation induced by cobalt feeding and a subsequent decrease when liver was added. In order to test the polycythemia producing potency of cobalt in dogs on milk diets we conducted the experiments described below. It was hoped that these experiments would serve also to throw some light on the controversial findings of Davis (5) and of Brewer (6).

EXPERIMENTAL. *Methods.* In the case of the adult dogs used in this study blood samples were obtained from the radial vein while blood from the younger animals was drawn from the jugular vein. In all cases 5 to 10 cc. of blood was drawn into the non-shrinking oxalate mixture described by Wintrobe (12). Hemoglobin, hematocrit and erythrocyte measurements were made within one hour after sampling. Hemoglobin concentration was measured by a method adapted to the Evelyn photoelectric colorimeter and the percentage volumes of packed red cells were determined by means of the Wintrobe hematocrit tube (13). Hayem's solution was used to dilute the blood for the erythrocyte counts which were made on a Spencer Bright-Line hemocytometer. Mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration calculations were made by the method suggested by Wintrobe (14).

Inhibition of hematopoiesis by cobalt feeding. The following experiments were carried out on four separate dogs at different times, but the methods used were comparable and the experiments can be described together. The dogs were all about one year of age and had received only milk with iron, copper and manganese from infancy. All of them had been cured of two successive anemias with copper and iron and were normal when the experiment was begun. Each dog was placed on a diet of milk alone and was bled periodically until a stable state of anemia was reached (Hb about 8 grams per 100 cc. blood). They were then given 4 mgm. daily of cobalt as a solution of cobaltous chloride. This was continued for two weeks without much apparent effect, except possibly to aggravate the anorexia already apparent due to the anemia. After two weeks of cobalt feeding, iron and copper were added at levels of 30 mgm. and 4 mgm. respectively daily. No beneficial effect was noted during the following two week period of iron, copper and cobalt feeding. In the case of dog 2, in which the effect was first noted, the iron level was increased to 60 mgm. daily, without apparent benefit to the dog. After two weeks of iron, copper and cobalt therapy, the supplements of the individual dogs were varied. Dog 2 was given 100 grams of whole dry liver and dog 4 was given 20 grams of liver extract (1:20 powder) in addition to the previous therapy. In each case an increase in appetite, weight and hemoglobin level ensued. The magnitude of the increases is seen in figure 1.

Dogs 6 and 13 were carried for a considerable period without any therapy in addition to the iron, copper and cobalt. Various supplements were

tried over a period of months and finally the dogs were brought to normal by the addition of liver fractions.

Production of polycythemia in adult dogs by cobalt feeding. The average blood picture of five adult dogs maintained on milk with added iron, copper and manganese is shown in table 1. The average hemoglobin level, erythrocyte count and hematocrit per cent of these dogs compare favorably with the averages found by Bruner and Wakerlin (15) for normal adult dogs. Addition of 3 to 6 mgm. cobalt per kilogram of body weight to the regular daily supplement of 30 mgm. iron, 3 mgm. copper and 3 mgm. manganese resulted in a large increase in blood values in the first few days

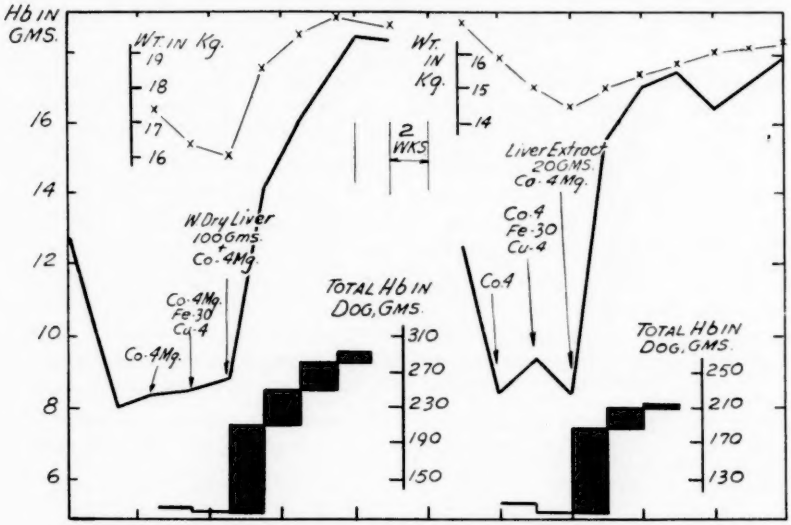

Fig. 1. The effect of liver and liver extract on anemia produced by bleeding and resistant to iron, copper, manganese and cobalt.

of cobalt feeding. The highest values obtained by this procedure were: hemoglobin 20.6, hematocrit 65, and erythrocyte count 11.6. Generally the blood values decreased gradually despite continued cobalt feeding until the blood picture was nearly normal at the end of three or four weeks. The fact that a clear-cut polycythemia was obtained during the first three weeks of cobalt feeding is shown in table 2. The calculated corpuscular values for the polycythemic bloods are probably not significantly different from those of the normal bloods.

The addition of 75 grams of fresh calves' liver, 25 grams of whole dried hog liver, 8 mgm. choline per kgm. of body weight, or 60 mgm. of crystalline vitamin C daily did not appear to cause a more rapid lowering in polycy-

themic blood values than naturally occurred over a period of 3 to 4 weeks. The addition of liver improved the general condition of polycythemic dogs as evidenced by weight gain and, if anything, caused the polycythemia to be maintained.

Effect of high levels of cobalt in young dogs. Considerable data have been obtained (7, 8, 9) on the normal rate of hemoglobin production in young dogs on milk diets with added iron, copper, manganese and traces of cobalt. These studies have revealed a slow prolonged rise to normal adult blood values during growth, a phenomenon common to most animal species.

TABLE 1

Blood picture of five adult dogs on a mineralized milk diet

Composite of a large number of analyses

MEASUREMENT	MEAN	MAXIMUM	MINIMUM
Erythrocytes, million per cmm	6.97	7.8	6.1
Hemoglobin, grams per 100 cc	14.29	17.5	11.5
Hematocrit, per cent	43.3	52	39
Mean corp. volume	62	83	55
Mean corp. Hb	21	26	17
Mean corp. Hb conc	33	34	27

TABLE 2

Typical average blood values in first three weeks of cobalt feeding in adult dogs maintained on milk plus iron, copper and manganese

INDEX	DOG 19	DOG 4	DOG 6
Erythrocytes, million per cmm	9.2	8.4	7.7
Hb, grams per 100 cc	18.1	19.8	16.9
Ht, per cent	55	58	52
Mean corp. volume	60	69	68
Mean corp. Hb	19.7	23.6	22
Mean corp. Hb conc	33	34	33

This apparent physiological lag phase may be largely independent of the nutrition of the animal and we desired to study the effects of high levels of cobalt during this stage. As the following experiments show, cobalt at levels which produce transient polycythemia in adult dogs, does not stimulate blood production in young dogs and is furthermore quite toxic.

Puppies were raised for this experiment in customary fashion on adequate levels of iron and copper and milk *ad libitum*. At 15 weeks of age dogs 26, 27, 29 and 30 were fed daily 80 mgm. of cobalt as cobaltous chloride in addition to 10 mgm. of iron and 2 mgm. of copper. Blood indices showed no significant change over a two week period of therapy. At 17 weeks of age

the iron supplement was raised to 30 mgm. daily and 2 mgm. of manganese was administered. No significant change in blood values was noted over two weeks of therapy. However, toxic symptoms began to appear at this point. Loss of weight, anorexia and vomiting were noted. The dogs were taken off therapy at 19 weeks of age.

At 20 weeks of age dogs 26 and 29 were fed daily 30 mgm. of iron, 2 mgm. of copper, in addition to 2 mgm. of manganese and 10 grams of brewer's yeast. After two weeks of therapy, the blood indices showed no significant changes but the dogs returned to normal in respect to daily weight gains and appetite. At 22 weeks of age dogs 27 and 30 were fed daily 30 mgm. of iron, 2 mgm. of copper and 2 mgm. of manganese in addition to 40 mgm. of cobalt. The dogs maintained their weight and appetite but the erythrocyte count fell from an average of 5.7 to 4.9 million per cubic millimeter of blood. Also at 22 weeks of age dogs 26 and 29 were fed daily 40 mgm. of cobalt in addition to iron, copper, manganese and yeast. No toxic symptoms were noted and erythrocyte counts remained relatively constant. At 24 weeks of age dogs 27 and 30 were given daily supplements of 10 grams liver extract (no. 343) in addition to 30 mgm. of iron, 2 mgm. of copper, 2 mgm. of manganese and 40 mgm. of cobalt.

No significant changes in the blood indices of the dogs were noted during the periods of high cobalt feeding. The general condition of the animals improved when the cobalt therapy was suspended. The yeast and liver additions were perhaps too small to cause any significant changes in the blood picture in the presence of cobalt. Data for one dog in each pair are shown in table 3.

Discussion. The results of our experiments on the polycythemia producing effect of cobalt cannot be safely compared with those of Davis (5) and Brewer (6) because of the difference in basal diets used. The general agreement is made, however, that polycythemia can be produced in adult dogs by cobalt feeding. Our results indicate that this is not true for young growing dogs and that in adult dogs the effect is transient.

The ability of liver to overcome cobalt inhibition to normal hematopoiesis in dogs made anemic by hemorrhage is of interest because the notion has long been held that liver contains a substance which is concerned with the maintenance of the level of erythrocytes in the blood. Anderson, Underwood and Elvehjem (16) have stabilized cobalt polycythemia in rats by the addition of liver and liver fractions to mineralized milk diets. Davis has attributed a polycythemia depressing action to liver, and more specifically to choline (11) and vitamin C (17). Verzar (18) and Zih (19) have shown the erythropoietic and erythropenic action of bilirubin in rabbits. Barron and Barron (20) found that ascorbic acid prevents cobalt polycythemia in rabbits and suggest, therefore, that ascorbic acid is a regulator of red blood cell production.

Other experiments support the view that physical factors are important, at least in initiating erythrogenesis. Gordon and Kleinberg (21) have shown that guinea pigs subjected to low pressures develop erythremia, and their experiments indicate that when the bone marrow is stimulated excessively, it possesses a momentum which carries it beyond its normal limits of erythrogenesis.

TABLE 3

Blood changes in young dogs on high levels of cobalt

DOG	SUPPLEMENT	AGE	WEIGHT	Hb	Ht	RED BLOOD CELLS	Hb IN DOGS	MEAN CORP. Hb	MEAN CORP. VOL.	MEAN CORP. Hb CONC.
		weeks					*grams*			
26	Fe 10 + Cu 2 + Co 0.1	13	5.3	14.3	41	6.1	60	23.4	67	35
	Fe 10 + Cu 2 + Co 80	15	6.0	12.8	39	5.9	61	21.6	66	33
	Fe 30 + Cu 2 + Mn 2 + Co 88	17	6.0	12.9	39	5.8	62	22.0	67	33
	Off therapy (toxicity symptoms)	19	6.7	12.5	39	6.6	67	19.0	59	32
	Fe 30 + Cu 2 + Mn 2 + yeast 10 grams	20	5.5	13.7	44	6.8	60	20.0	65	31
	Fe 30 + Cu 2 + Co 40 + Mn 2 + yeast 10 grams	22	6.8	12.6	39	6.5	68	20.0	60	32
		23	7.7	12.2	40	6.2	75	20.0	65	30
		24	7.1	13.7	41	5.9	78	23.0	70	33
30	Fe 10 + Cu 2	13	6.0	13.5	39	5.6	65	24.0	70	35
	Fe 10 + Cu 2 + Co 80	15	6.7	13.9	38	5.2	74	27.0	73	37
	Fe 30 + Cu 2 + Mn 2 + Co 80	17	6.3	15.2	44	7.1	77	21.0	62	35
	Off therapy (toxicity symptoms)	19	5.8	16.0	47	7.6	74	21.0	62	34
		20	5.6	15.3	47	6.4	69	24.0	74	33
	Fe 30 + Cu 2 + Mn 2 + Co 40	22	6.8	12.9	38	5.3	69	24.0	72	34
		23	7.5	12.1	37	5.0	73	24.0	74	33
	Fe 30 + Cu 2 + Mn 2 + Co 40 + 10 grams L.E. #343	24	7.4	13.7	41	4.9	81	28.0	84	33
		25	7.35	14.7	44	5.4	86	27.0	81	33
		26	8.2	12.9	43	5.4	85	24.0	80	30

The failure of copper and iron to initiate hematopoiesis in phlebotomized dogs fed cobalt, and the profound response to liver strongly supports the idea that liver contains something other than iron and copper which is intimately concerned with stimulation of the blood forming centers.

Whether this substance is generally required in nutrition or whether it is of hormonal nature is not known. Preliminary evidence has been obtained that the pernicious anemia principle from liver is not involved. Vitamin B_6 appeared to cause some stimulation, but the response was not as great as that obtained with liver or liver extract (1:20 powder).

The importance of the ration in this type of study cannot be overestimated and it is questionable whether the results obtained with milk diets can be extended to other adequate diets. Although milk plus iron, copper and manganese supports a high rate of hematopoiesis in normal dogs, it differs in blood building properties from liver on a quantitative basis and possibly on a qualitative basis as well.

SUMMARY

1. An inhibition of the normal hematopoietic response to iron and copper feeding was observed in dogs made anemic by hemorrhage and fed cobalt prior to the addition of iron and copper. Hematopoietic activity was resumed on the feeding of whole dry liver or liver extract. The compound(s) in liver which cause this profound hematopoietic response have not been determined.

2. A temporary polycythemia was produced in adult dogs by feeding 3 to 6 mgm. of cobalt per kgm. of body weight in addition to the usual mineralized milk diets.

3. High levels of cobalt had a toxic effect on young growing dogs and little apparent effect on the blood picture.

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SHOCK FOLLOWING VENOUS OCCLUSION OF A LEG[1]

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In the course of experiments in which attempts were made to produce chronic edema the procedure of ligation of the iliac vein with injection of various materials distally into the vein was initiated. In a number of instances marked edema of the legs developed and associated with this the animals died within 24 hours, sometimes within 4 to 6 hours. The possibility of shock as the cause of death suggested itself and this was investigated in a series of 8 dogs.

METHOD. The dogs were anesthetized with ether. The common and internal iliac veins were ligated aseptically on one side using a retro-peritoneal approach; this was followed by the injection distally into the external iliac vein of 12 to 15 cc. of an autoclaved 1:20 suspension of lampblack in physiological saline solution. The duration of the anesthesia was between 30 and 45 minutes. Several types of measurements were made at frequent intervals and compared with control observations made before the anesthesia and operation had been undertaken: *a*, arterial blood pressure measurements, using the Hamilton needle manometer technique (1), the needle being inserted in the contralateral femoral artery; *b*, heart rate, calculated from these records; *c*, hematocrit determination, using the method recommended by Scudder (2); however, blood was drawn from the femoral artery because of the difficulty of obtaining blood from the collapsed veins late in the course of the experiments; *d*, total plasma proteins, determined by the Kjeldahl method (3); *e*, rectal temperature; *f*, respiration, by timing with a stopwatch; *g*, measurements of the limb circumference of both hind legs as a crude guide to the development of edema. After the death of the animal, each of the hind limbs was disarticulated at the symphysis pubis and hip-joints, being sure to include the gluteal muscles, and then weighed.

RESULTS. The leg with the occluded veins became markedly enlarged and colder than the contralateral limb. The animals became listless and quiet, even during the manipulations of taking the various readings. Respirations usually increased in rate, especially toward the end when they became shallow and panting. Retching and vomiting usually occurred

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terminally, as well as cycling and spasmodic movements. Death was respiratory, cardiac standstill being delayed for several minutes.

There was no significant change in the rectal temperature throughout the experiment.

The changes in the total plasma protein, from blood taken at the beginning and at the end of the experiment, were not marked and probably within the experimental error of the determination. In 2 dogs total protein determinations were made at frequent intervals during the course of the experiment. Since only slight alterations were observed, it would appear that the capillaries of the limb with occluded veins lost their impermeability to the blood proteins early. The fluid lost from the blood therefore appears to be practically a plasma.

The blood pressure fell progressively to shock level; at first this affected the systolic and pulse pressure more than the diastolic. In part this was due to the marked acceleration of the heart, in part to the decrease in blood volume reaching the heart due to a loss of plasma as shown by the hematocrit. In the two animals which survived longer, there was a tendency for the blood pressure to rise in the last hour before death (fig. 1).

The heart rate increased after the operation and remained elevated until shortly before death when a final slowing occurred. In three of the animals slowing was noted to occur in the last hour of life.

In all instances there was a progressive hemoconcentration as measured by the hematocrit, and usually there was a terminal tendency for this to return toward normal. This hemoconcentration indicates a loss of plasma fluid into the edematous leg. The factors involved in the final decrease in the hematocrit appear to be:

1. A movement of fluid from other extracellular spaces to replace that lost into the edematous limb.

2. The increase in tissue pressure in the edematous leg lessening the movement of plasma out of the blood stream.

3. The development of hemorrhages.

The increase in the weight of the edematous leg over the contralateral one was remarkable as shown in table 1. The values range from 4 per cent to 6.1 per cent of the body weight. It is significant to note that there was a rough inverse ratio between the relative amount of fluid lost in the leg and the duration of life after operation. The loss of fluid thus amounted to approximately 50 per cent to 75 per cent of the usually accepted blood volume. It follows that this would cause not only the blood hemoconcentration found and a sharp drop in the circulatory blood volume but would of necessity draw upon the extracellular fluid reservoirs. This implies that one is dealing with a state of body dehydration and the conditions resulting from it.

Figure 2 represents one of the experiments in which muscle biopsies were

taken from the edematous leg. The histological sections are shown in figure 3. From such studies, the following sequence appears to have occurred. At first, slight distention of the capillaries was revealed with the presence of edema fluid in their vicinity. Later, the distention of the

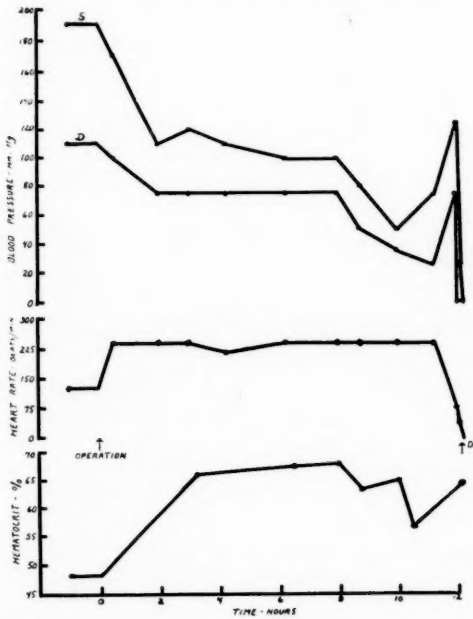

Fig. 1

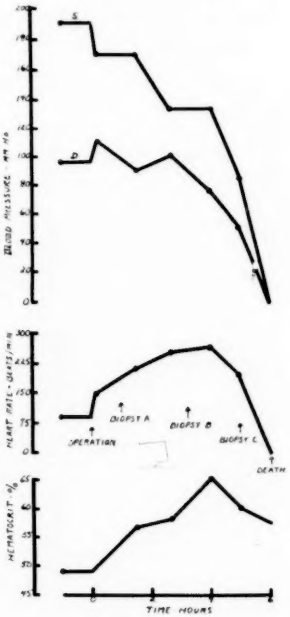

Fig. 2

Fig. 1. A typical chart of events following almost complete venous occlusion in one hind limb. S = systolic blood pressure; D = diastolic blood pressure. Weight of dog, = 12.7 kgm. Weight of disarticulated left leg (with venous occlusion), = 1.567 kgm. Weight of disarticulated right leg (control), = 1.064 kgm. Difference in weight, = 0.503 kgm.

Fig. 2. Another typical experiment, conventions as in figure 1. Weight of dog, = 13.6 kgm. Weight of disarticulated left leg (with venous occlusion), = 2.305 kgm. Weight of disarticulated right leg (control), = 1.568 kgm. Difference in weight, = 0.737 kgm. Muscle biopsies were taken from the leg with venous occlusion at the times indicated, and these, as well as sections taken at autopsy, were examined histologically.

capillaries and veins became more pronounced. When the dog was in shock, red blood cells appeared between the individual muscle fibers and in the intermuscular septa, and recent thrombi containing numerous lampblack particles were seen in the veins. The hemorrhages became progressively greater until death supervened.

TABLE 1

WEIGHT OF ANIMAL	INCREASE IN WEIGHT OF EDEMATOUS LEG OVER CONTRALATERAL ONE		DURATION OF LIFE AFTER OPERATION
kgm.	*grams*	*per cent body weight*	*hours*
10.9	668	6.1	$4\frac{1}{2}$
9.5	570	6.0	5
11.8	576	4.9	$5\frac{1}{4}$
13.6	737	5.4	6
13.2	702	5.3	$6\frac{1}{2}$
15.4	750	4.8	$9\frac{1}{2}$
10.5	458	4.3	10
12.7	503	4.0	$13\frac{1}{2}$

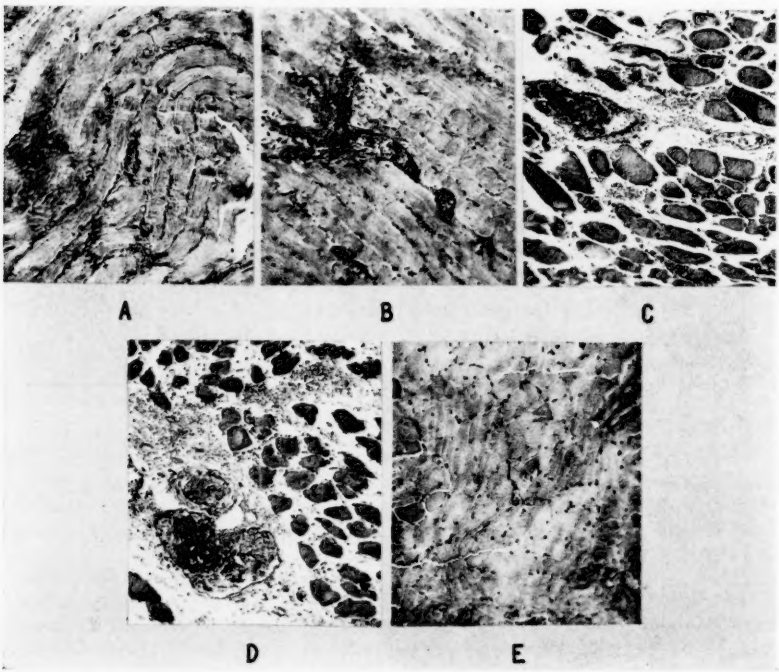

Fig. 3. Photomicrographs of biopsies of leg with venous occlusion, *A*, *B*, *C*, and of autopsy section from this leg, *D*, and from the contralateral control leg, *E* (Weigert's iron hematoxylin-eosin stain; × 110). Segment *A* shows in upper left hand corner slight dilatation of capillaries with surrounding edema. Segment *B* shows marked dilatation of the capillaries and veins. Segment *C* shows red blood cells between the individual muscle fibers and in the intermuscular septa; the vein contains a recent thrombus in which are numerous lampblack particles. Segment *D* shows even more hemorrhages between the muscle fibers and in the intermuscular septa, as well as a recent thrombus in the vein. Segment *E* shows no histopathologic changes.

Post-mortem examinations of the other organs showed several definite changes. The suprarenal glands revealed varying degrees of hyperemia and occasionally microscopic hemorrhages. These hemorrhagic areas were confined to the zona fasciculata and contained many polymorphonuclear leukocytes. There was marked dilatation of the central veins and sinusoids of the liver, and occasionally hyperemia of the capillaries of the lung and intestinal mucosa. The brain removed from one of the animals was very pale, and there was no gross or microscopic evidence of capillary dilatation or hemorrhage. The remaining organs presented no significant changes.

Discussion. These experiments illustrate that an extremely large amount of fluid can be accumulated in a limb when its venous channels are fairly completely occluded; sufficient, in fact, to cause hemoconcentration, a drop in blood pressure to shock levels and symptoms and signs of shock terminating in death. It is possible that on rare occasions an extensive thrombophlebitis of the common iliac vein in man, developing very rapidly, may lead to death in a like manner. Such appeared to be the case in a patient recently reported by Phemister (4). A woman developed a rapidly increasing swelling of one leg and died within 24 hours in shock. At autopsy a fresh thrombophlebitis completely blocking the common iliac vein and partly blocking the inferior vena cava was found. From measurements of the lower limbs, it was calculated that the volume of the limb with the thrombosis was about 4 liters greater than the contralateral one so that the immediate cause of death appeared to be due to the great loss of plasma into the occluded limb.

The procedure carried out in these experiments seems to offer a simple, certain method of inducing a state of shock and so lends itself readily to the utilization of the study of the sequence of events that occur and as a simple procedure to test the efficacy of some forms of suggested shock therapy.

It illustrates the magnitude of fluid loss from the rest of the body which can be accomplished by such a procedure and lends support to the most widely accepted view held at present, namely, that such localized fluid loss is the primary, or, at least, the most important factor involved in the production of shock (5). Apparently, in these experiments it was the fluid loss per se that established the chain of events leading to shock and early death.

The dynamics involved in the accumulation of the fluid locally are apparent. The ligation of the major veins of the limb by themselves, we have found, does not cause edema, or only a slight and transient edema. Apparently there is a widespread reserve of collaterals that quickly compensates for the occlusion and permits the blood drainage to be quickly reestablished. The injection of lampblack seems to form foci for the forma-

tion of thrombi resulting in the plugging of the majority of these collaterals. As a consequence, drainage of blood is slowed down markedly. The blood vessels in the leg, therefore, change quickly from a hydraulic system with blood in motion to a practically hydrostatic one with little flow. This implies that the gradient of pressure normally existent from the arteries to the veins disappears to a large extent. As a result, the pressure in the capillaries will tend to approach the pressure in the arteries and this would cause the hydrostatic factor to increase over the oncotic and thus lead to the pouring out of fluid into the leg tissues. Two circumstances would tend to limit this fluid escape. The first is the increase in tissue pressure; the second, the increase in oncotic pressure as fluid escapes and blood proteins do not. However, the stasis itself would quickly alter the permeability of the capillaries and so permit the protein also to escape, as our blood protein studies suggest. In fact, this damage to the capillaries soon becomes sufficient to allow the escape of blood and so lead to the hemorrhages, which were actually found to be extensive.

As a result of this fluid loss the entire picture of shock developed and led to the animals' death.

SUMMARY

1. Nearly complete venous occlusion of a hind limb of the dog leads to shock which terminates fatally.

2. This procedure offers a simple way of studying the course of shock and the utility of some of the proposed therapeutic agents to counteract shock.

3. The mechanism appears to be the marked loss of fluid into the leg, at first plasma and later whole blood, which amounts to from 4 to 6 per cent of the body weight. This loss is brought about first by an increase in the capillary hydrostatic pressure of the occluded limb soon aggravated by loss of capillary permeability.

4. These experiments tend to support the view that the primary mechanism in shock is the local loss of fluid from the blood.

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Addendum. Since the communication was sent to press 5 more animals were tested and in general the relation of duration of life after the operation to the percentage loss of body fluid into the limb followed the same trend evidenced in table 1.

THE ACTION OF EXERCISE ON KETOSIS

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In recent years evidence has been accumulating that the ketone bodies are, on occasion, an important step in the catabolism of fat (1, 2). There is much support for the view that the liver at certain times partially oxidizes fatty acids to form these ketone bodies which are then supplied to the other tissues of the body as an important fuel (3, 4). A large fraction of the energy turnover of the muscles may under certain conditions be supplied by oxidation of these substances (1, 5). It would seem likely then that individuals in a state of ketosis would show a decrease in its intensity (which has ordinarily been measured by urinary ketone excretion) as a result of exercise. This has not been found to be entirely the case for periods of exercise lasting an hour or more (6, 7). These seemingly contradictory findings may be reconciled if we can accept the assumption already stated in papers from these laboratories (1) that muscular activity not only causes increased burning of these bodies but also increased production of them by the liver.

If these two effects are produced at the same time they would tend to balance each other and it would be very difficult to investigate them separately. If they are not simultaneous,—one being delayed—, it should be possible to follow the successive opposite effects. The increased utilization of ketone bodies by working muscles is immediate as is evident from the observations of Blixenkrone-Moller (5) on perfused isolated extremities and of Drury and Wick (8) on the intact subject. If there is an increased output by the liver and it has a delay of over half an hour it should be possible to follow it since it should give a post-exercise increase in the blood ketone curve. With the human subject we experienced difficulty in producing a "steady" ketosis state on which we could study this delayed effect of exercise. One can easily produce a definite ketonemia by a fast of 20 hours, but at this time the level is not constant, but has a distinct, though not necessarily steady upward gradient. Over a three day period this averages 0.82 mgm. per cent per hour (9). Our subject showed a gradient of 2.2 mgm. per cent per hour between the 10th and 20th hours. Under these conditions it is easy to study mechanisms which lower the

ketone content of the body, but it is difficult to follow those which increase it. One is forced to attempt to determine whether the post-exercise blood ketone curve has a steeper gradient than a control curve for the subject during the same period of fasting.

HUMAN EXPERIMENTS. In all of our human experiments we produced ketonemia by having the subject go without food for at least 15 hours. This was easily carried out by having him take no food after the evening meal of the previous day and then starting observations at 9 a.m. or later. At this time the blood ketone level is beginning to rise and continues to do so for many hours (8). We then had the subject exercise or rest for varying periods to see how this upward gradient of blood ketones would be affected during the exercises and during the period which followed. Blood ketone

TABLE 1

DAY 1		DAY 2	
Time	Blood ketones	Time	Blood ketones
	mgm. per cent		*mgm. per cent*
9:25 a.m.	1.8	9:15 a.m.	1.2
Walking		Rest	
10:50 a.m.	2.5	10:20 a.m.	1.2
Walking		Rest	
11:50 a.m.	1.6	11:05 a.m.	1.3
Rest		Walking	
1:10 p.m.	6.0	1:10 p.m.	3.4
Rest		Rest	
3:15 p.m.	8.9	2:15 p.m.	4.8

determinations were done by the method of Barnes and Wick (10) and are expressed as total ketone bodies.

Light exercise. The first exercise studied was walking. This was studied on two separate days a week apart. Every attempt was made to keep conditions identical on the two occasions (character of food on previous day, times of eating and sleeping) and the only thing that was varied was the times of walking and resting.

Although the results of the first day might suggest an increase in utilization during walking with an increased production in the supervening rest period, the second day shows the same general upward trend at any comparable period regardless of whether the subject rested or walked, or had been walking just before. We may conclude then that if walking does increase the utilization of ketone bodies it does so to but a small extent, and this small increase would be compensated for by increased production by the liver.

Moderate exercise. A heavier form of exercise was next studied—tennis playing. Table 2 shows the results of our first experiment with this.

It is apparent that playing tennis for this period has little demonstrable effect on the course of the blood ketone level. Its steadily rising trend may have been slightly flattened during playing. We, therefore, examined the effect of this form of exercise when continued for a longer period. Table 3 gives the results together with a control day on which conditions were identical except for exercise.

It would seem that exercise might have leveled out the upward trend of the blood ketones during the first hour and a half, but not after this. The rise between 11:04 and 12:20 may represent increased production by

TABLE 2

TIME	BLOOD KETONES
	mgm. per cent
3:20 p.m.	5.5
4:20 p.m.	6.4
Tennis playing	
5:10 p.m.	6.6
7:05 p.m.	12.7

TABLE 3

EXERCISE DAY		CONTROL DAY	
Time	Blood ketones	Time	Blood ketones
	mgm. per cent		*mgm. per cent*
9:37 a.m.	6.7	9:40 a.m.	6.0
Tennis		11:40 a.m.	8.2
10:13 a.m.	5.9	1:00 p.m.	19.7
Tennis			
11:04 a.m.	6.3		
Tennis			
12:20 p.m.	12.8		
Rest			
2:05 p.m.	19.5		

the liver. The differences from the control period, however, are so slight that it appears again that in moderate exercise one cannot demonstrate increased utilization of ketone bodies followed by increased production of these substances. Both may be increased but if so they offset each other very closely.

Heavy exercise. With a higher rate of exercise—running steadily at 10 miles per hour for 20 minutes—we obtained a definite decrease in the blood ketone level. This drop was followed by a sharp rise during the period after the exercise. See tables 4 and 5.

The examples show the drop during the heavy exercise and the sharp rise immediately after. The control days, however, have periods during

which the blood ketone level rises just as steeply. We can conclude then that a short bout of heavy exercise can use up ketone bodies faster than they are being formed by the liver. It is not possible to tell whether the succeeding rise due to liver production is greater than what would ordinarily occur because the control periods show at times rises just as steep.

RAT EXPERIMENTS. It seemed to us that the occurrence of these sharp rises in ketonemia on the control days make it very difficult to find out whether there is an over-production of ketone bodies by the liver after exercise in the human. We decided then to study an animal that had a

TABLE 4

CONTROL DAY		EXERCISE DAY	
Time	Blood ketones	Time	Blood ketones
	mgm. per cent		*mgm. per cent*
11:40 a.m.	8.2	3:40 p.m.	11.8
1:00 p.m.	19.7	4:40 p.m.	15.7
4:00 p.m.	22.9	Exercise	
		5:02 p.m.	9.6
		6:10 p.m.	16.2
		7:15 p.m.	18.8
		8:30 p.m.	20.3

TABLE 5

CONTROL DAY		EXERCISE DAY	
Time	Blood ketones	Time	Blood ketones
	mgm. per cent		*mgm. per cent*
12:10 p.m.	2.1	2:25 p.m.	4.3
3:20 p.m.	3.5	3:25 p.m.	7.7
5:25 p.m.	8.0	Exercise	
		3:45 p.m.	3.7
		5:00 p.m.	7.5

more slow and gradual rise in blood ketones during simple fasting (11, 12). The rat, which as an example may have an average rate of rise in the blood ketone level of 0.2 mgm. per cent per hour between the zero and ninety-sixth hour of fasting, satisfies this requirement. Even more important for the present problem, it is possible to fast these animals for periods adequate to arrive at a state in which the blood ketone level reaches a plateau and remains practically constant (13).

We made observations on 3 series of rats. For each series we selected a group of adult rats of common origin, of the same sex, approximately the same weight and within thirty days of the same age. They were all fasted

the same length of time, and then divided into two parts—one for the exercise and one for the control. The rats of the former group were given a short period of strenuous exercise (swimming) and at set times thereafter sub-groups of six were sacrificed for individual blood ketone determinations upon arterial blood. Similar sub-groups of six of the unexercised group were sacrificed at set times for control blood determinations. In all cases the liver glycogen content was also determined. Male rats were used in experiments A and B. In experiment A the rats (average body weight 281 grams) were used 48 hours after their removal from the stock diet ("Tioga dog Pellets", protein, 23; fat, 4; fiber, 4; ash, 12.5; moisture, 8.5; nitrogen free extract, 48). The rats in experiments B (average body weight 300 grams) and C (average body weight 202 grams) were on a low protein diet (13) for 15 and 16 days respectively and then fasted 3 days before using. Such a diet leads to a high fasting concentration for the blood acetone bodies (14).

The animals were exercised by swimming them in water (32°C) for five 2 minute periods with 1 minute rest between periods. With this amount of exercise the rats are completely exhausted and further swimming is generally impossible. The blood ketones were determined by the method of Barnes and Wick (10). Oxalated blood specimens were obtained from the abdominal aorta after the animals were anesthetized with sodium pentobarbital. Glycogen determinations were carried out on the livers according to the method of Good, Kramer and Somogyi (15).

The results are given in figure 1. Each point in a given experiment represents the average of the determinations for a group of six rats. The rats used in experiment A were evidently not fasted long enough to give a very high beginning blood ketone level, so the drop after exercise is not large. The subsequent over-production is quite definite. In the next series (B) we, therefore, made sure of an adequate beginning blood ketone level by feeding of a low protein diet and a longer fast. We also extended the time of post exercise observations. In this series we obtained a marked drop in blood ketone level immediately after the exercise, with a return to the control level one hour later. This is followed by an over-production phase, so that the level is much higher than the control two hours thereafter. At six hours after exercise the level has come back to that of the controls. In series C we produced a still higher beginning level, extended further the post-exercise period and determined the blood ketones every hour after the exercise. The results are even more pronounced than in experiment B. Two hours after exercise the over-production phase has not only made up for the immediate drop but has taken the level to well above the control. From then the level continues to rise slightly until the fifth hour. At the sixth and seventh hours the level is back to the control.

The changes in liver glycogen content pictured in figure 1 represent

weighted averages of the data for experiments A, B and C in which the changes were of the same general nature. The liver glycogen level was fairly low to begin with because the animals were fasting. During the brief exercise period it fell to a really low level and remained there throughout the period of observations. This is not surprising for in these glycogen depleted rats the only possible source of additional carbohydrate would be from protein catabolism. Although it has often been questioned, the old experiment of Pettenkofer and Voit (16), who found that in starvation exercise did not increase protein metabolism, has not been disproved. The energy for the exercise must be supplied by an increased metabolism

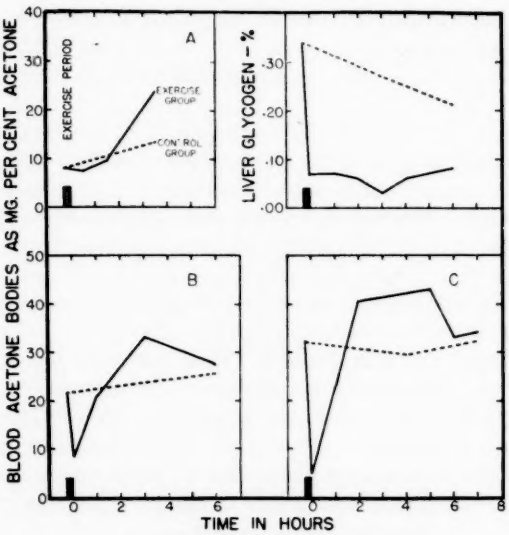

Fig. 1

of fat. The ketone bodies during exercise, when fasting, serve in part at least the functions of glucose during exercise in the fed organism (17–20).

Discussion. The results with the rat show that we can demonstrate clearly both increased utilization, and subsequent over-production of ketone bodies as a result of strenuous exercise in this animal. Heavy exercise also gives clear evidence of increased utilization with the human, and it is likely that a subsequent over-production would be clearly shown if we had fasted the subject until he had reached the blood ketone plateau. Evidently, demonstration of the two processes would be difficult for lower rates of exercise. Here the intensities of the two effects are less and in order to get the definite drop in blood ketone level that is needed to prove

increased utilization the exercise has to be continued for such an extended period that we get overlapping of the increased-production mechanism, and in this way the results of the two actions are balanced and consequently hidden.

Our results solve the problem presented by the finding of Blixenkrone-Moller that increased work by isolated muscles increases utilization of ketone bodies whereas increased muscular activity by the intact ketonic animal does not decrease the urinary excretion of ketone bodies in the 24 hour period. If the rate of exercise is high there is a reduction of ketones in the body and urine at the time, but this is compensated for by over-production during a period of several hours following the exercise, so that the total excretion during the two periods is little changed from control periods. It is reasonable to assume that the same mechanisms account for the absence of effect of less strenuous exercise continued for longer periods. Here the supervening increased production would commence before the increased utilization due to the exercise had continued long enough to have had a demonstrable effect. Although we can only get a separation of the two effects with short strenuous exercise, we have every reason to believe that they occur in milder forms of activity, here co-existing and offsetting each other.

A word should be said as to the possible mechanism of the action of exercise on ketosis. The initial fall in the ketone body level of the blood which results from exercise is most reasonably explained by an increased rate of utilization. What then of the subsequent overproduction? In severe exercise epinephrine secretion is abundant (20, 21) and causes an increase in glycogenolysis when carbohydrate for such is available. When there is a lack of carbohydrate, epinephrine increases ketone body formation (22). This may well be the cause of the over-production of ketone bodies during and immediately after severe exercise. Epinephrine may be an agent for increasing the production of ketone bodies when there is a condition that may require a lot of them (carbohydrate lack), similar to the way in which it increases glucose production when there is a need for it and an ample supply of glycogen is available.

SUMMARY

1. With rats, in a state of ketosis, a short bout of heavy exercise causes an immediate drop in the blood ketone level. During a period of three to four hours thereafter there is a phase of over-production of ketone bodies so that the blood values for exercised animals go to higher levels than in controls.

2. These results support the view that in ketosis states exercise increases the oxidation of ketone bodies and also causes the liver to produce them at a higher rate.

3. In man these changes were not demonstrated for light exercise. The drop during heavy exercise was obtained.

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FETAL ASPIRATION OF AMNIOTIC FLUID[1]

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The classical concept that the fetus in utero is apneic has from time to time been challenged and some investigators have declared that respiratory movements occur regularly throughout the latter part of prenatal life and represent normal physiological adjustments. On the basis of observations made on rabbits, Snyder and Rosenfeld (1) believe that the fetus in utero has a definite respiratory cycle and that amniotic fluid is normally drawn into the alveoli of the fetal lungs. They hold further that intra-uterine breathing is of functional significance in the development of the normal lung and serves to assist in the dilatation of the alveoli and elastic passages of the future air passages. Contrary to this conception are the observations made by Windle and his associates (2) on fetal goats, guinea pigs and cats. These investigators conclude that respiratory movements occur *in utero* only when abnormal physiological conditions (such as anoxia or hypercapnia) occur in the fetus. Clinical observations on the human fetus and new-born have led most obstetricians to consider the excessive inspiration of amniotic fluid as a predisposing factor toward pneumonia or other respiratory infections in the new-born. A high proportion of autopsies on new-born report the presence of amniotic sac contents in the lung. Thus, no one doubts that mammalian fetuses are capable of performing respiratory movements. However, the physiological normality of this process has not been established. In fact, there is clinical evidence that the aspiration of amniotic fluid into the alveoli of the lungs may result in death.

The following experiments were performed on rats in an attempt to determine the effect of inspiration of amniotic fluid on viability.

EXPERIMENTAL. On the nineteenth or twentieth day of pregnancy, the

[1] Grateful acknowledgment is made to Mr. Theodor Chernikoff for technical assistance in these experiments. This work was aided by a grant from the Research Board of the University of California. Clerical assistance in the preparation of these materials was provided by Work Projects Administration (O.P. 65-1-08-62, Unit A-8).

maternal rat was anesthetized with ether and the uterus exposed through a mid-line incision. One-half cubic centimeter of a warm sterile suspension of carbon in physiological saline solution was injected into the amniotic sac of each fetus directly under the fore leg. Care was taken to avoid injury to the placenta or to any of the uterine vessels when making the injection. After replacing the uterus, the animal was sewed up and delivery followed within 24 to 48 hours. All animals delivered dead were immediately removed and autopsy performed. All animals born alive were killed after 24 hours and autopsied. All lungs were removed and examined for the presence of carbon particles. When the lungs appeared pink or light grey, histological sections were prepared and a microscopic examination was made for the presence of carbon particles in the alveoli.

TABLE 1

	CARBON INJECTIONS		SALINE INJECTIONS
	Experiment I	Experiment II	
Number of fetuses injected	349	182	162
Number born dead	258	144	40
Percent of total injected born dead	69*	79†	25
Number dead within 24 hours (including still births)	279	166	68
Total mortality rate (per cent)	80	91	42
Animals living to 24 hours	70	16	87
Per cent of animals surviving at 24 hours with carbon in lungs	4	13	
Per cent of total animals injected living at 24 hours	20	9	58

* One hundred per cent of the 258 still-borns showed carbon in lungs.
† Ninety-three per cent of the 144 still-borns showed carbon in lungs.

In experiment I, 349 fetuses were injected with a suspension of carbon prepared by centrifuging a 1:1 dilution of Higgins' India Ink at 2,200 r.p.m. for 8 hours. The supernatant fluid was poured off, diluted with an equal volume of water and again centrifuged. This procedure was repeated twice until the ink was diluted to approximately one-quarter its original concentration.

In experiment II, a suspension of lamp black in 1 per cent gelatine was used for the injection. The carbon suspensions produced in this manner were non-toxic when injected intraperitoneally into 21-day-old rats.

A similar series of animals was run in which normal saline alone was injected into the amniotic sac.

RESULTS. The results of the experiments are shown in table 1. In experiment I, 69 per cent of the 349 fetuses injected were still-born.

In experiment II the proportion of still births was 79 per cent.

In both experiments carbon particles were found in the lungs of practically all of the still-born animals (100 per cent in expt. I and 93 per cent in expt. II). If the animals which failed to survive 24 hours after birth are included, the mortality rate rises to 80 per cent in experiment I and 91 per cent in experiment II. Of the 86 animals which were still alive 24 hours after birth, only 5 (or 6 per cent) showed any trace of carbon particles in the lungs on autopsy.

When normal saline was injected into the amniotic sacs, only 25 per cent of the animals were still-born, and the total mortality rate at twenty-four hours was only 42 per cent, as compared with 80 per cent and 91 per cent in the carbon injection experiments.

DISCUSSION. The results of these experiments indicate clearly that aspiration of large amounts of an amniotic fluid containing solid particles is fatal to the fetus. It may be that the stimulus to the aspiration was the anoxia and hypercapnia resulting from the operative procedures. However, the normal animals, as evidenced by their survival to 24 hours after birth, had inspired no amniotic fluid (or at best very little) under the same conditions, since their lungs showed no carbon particles.

Since the mortality rate[2] was 42 per cent when saline was injected, it is clear that death of the fetuses in experiments I and II cannot be attributed entirely to the presence of foreign particles (carbon) inspired into the lungs with the amniotic fluid. The high mortality rate found with the saline injections means that under the abnormal fetal conditions produced by the experimental procedure, amniotic fluid may be aspirated in sufficient amounts to result in the death of the fetus.

SUMMARY. A suspension of carbon particles was injected in the amniotic sacs of 431 rat fetuses one to two days *pre partum*. Approximately 75 per cent were still-born and only 15 per cent were living 24 hours after birth. In contrast, 58 per cent of a group of 162 fetuses in which normal saline was injected into the amniotic sac under similar conditions were living 24 hours after birth. Over 90 per cent of the still-born animals showed carbon particles in the lungs when autopsied. Since some animals did survive 24 hours after birth and showed no carbon in the lungs, it cannot be contended that aspiration of amniotic fluid is a normal physiological process. The high incidence of still-births in both series leads to the conclusion that the excessive respiratory stimulation resulting from the fetal anoxia and hypercapnia attendant on the anesthetic and operative procedure caused the animals to inspire amniotic fluid.

[2] The expected still-birth rate in rats is given at 2 per cent by Donaldson.

CONCLUSIONS

1. Not all fetuses aspirate amniotic fluid into the lungs even under adverse physiological conditions.

2. Excessive inspiration of amniotic fluid will result in the death of the fetus.

3. It is highly improbable that amniotic fluid is normally drawn into the alveoli of the lungs in the fetus.

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THE TRANSPORTATION OF ABSORBED LIPIDS[1]

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In 1891 Munk and Rosenstein (12) reported the recovery from a chylous fistula of 60 per cent of the fat ingested by the subject. Since that time this figure has acquired an importance out of all proportion to the character of the experimental work upon which it was based, the assumption having generally been made that it actually represents the fraction of ingested fat absorbed into the circulation via the lymph. This has never been confirmed, Eckstein (5), for example, being able to recover from thoracic duct lymph only 4 to 21 per cent of the fat administered to dogs. Both in the original work and in subsequent attempts to confirm it, values have been referred to administered rather than absorbed fat, the fraction of the administered fat that was actually absorbed not having been determined. Hence, it is possible that *all* of the fat that was absorbed entered the lymph.

By studying the changes in blood lipid concentration before and after ligation or cannulation of the thoracic duct or of the portal blood before and after administering lipids, many attempts have been made to account for the 40 per cent that Munk failed to recover from the lymph. The results are inconclusive.

In the work reported here, we have measured the actual amount of administered lipid which was absorbed and determined the fraction of this that appeared in the thoracic duct lymph. We have also measured the changes in the composition of the portal and femoral blood that occurred simultaneously. As a control the effect of the administration of non-lipid material on the lipid content of blood and lymph was also studied.

EXPERIMENTAL. Well nourished young dogs weighing from 14 to 18 kgm. were used. From a week to ten days prior to the experiment a permanent cannula, as described by Horine (7), was placed on the portal vein, so that repeated samples of blood could be obtained without the

[1] Aided by a grant to the Vanderbilt University School of Medicine from the Division of Medical Sciences of the Rockefeller Foundation.

[2] The data are taken from the thesis of J. Maxwell Little presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Vanderbilt University, 1941.

necessity of an open abdomen. The animals received their last food from 36 to 48 hours before the experiment was begun. They were anesthetized with either sodium barbital or Dial (Ciba) with urethane. An incision was made on the left side of the neck that exposed the external and internal jugular veins. The external vein was ligated distal to its junction with the internal jugular vein which was also ligated. The left subclavian and left innominate veins were ligated as well as several small veins emptying into the common jugular vein. In this way it was possible to cannulate the common jugular vein and collect lymph from the thoracic duct. In each case the course of the left thoracic duct and its several mouths, which were frequently found, was determined, so that the above ligations would not interfere with the flow of lymph.

A similar incision was made on the right side and all of the vessels entering the external and common jugular and innominate veins were ligated as well as the right thoracic duct. In this way entrance of lymph into the blood from the right duct was prevented, while maintaining an adequate venous return from the head.

All lymph flowing was collected for one hour and control samples of portal and femoral blood were drawn, after which the animal was given 100 cc. of milk or cream. To avoid the uncertainty due to the emptying time of the stomach, this was injected directly into the duodenum, exposed by a small incision made in the mid-line of the abdomen, which was immediately closed. The milk was skimmed milk which had been centrifuged to remove the residual fat and yield a fluid which was nearly lipid free but still similar to the cream in other respects.

Lymph was collected continuously throughout the experiment in tubes containing 0.1 cc. of a 3 per cent heparin solution. The collections were divided into arbitrary periods representing one hour intervals for the first two hours and two hour intervals thereafter. The volume of lymph for each period was recorded and aliquots were taken for extraction. Blood samples were drawn at the same time intervals and were immediately centrifuged and aliquots of plasma were extracted.

Throughout the experiment 50 to 100 cc. portions of normal saline were injected subcutaneously to replace approximately the volume of fluid withdrawn as lymph and blood. The experiment was terminated between the eighteenth and twenty-fourth hour after the injection of cream or milk. In some cases the flow of lymph ceased before the experiment ended, but in no case was this due to clotting. The flow of lymph was never stopped more than a few minutes by the rather infrequent clots which formed.

The animals were autopsied in each case to verify the location of the Horine cannula and to inspect the gastro-intestinal tract. In no case was there evidence of cream or milk in the lower part of the ileum. In

those animals receiving cream the stomach and small intestine were removed and thoroughly washed with several portions of distilled water. These washings were collected, and examined for lipids. In order to determine the efficiency of this recovery procedure, three experiments were performed in which 100 cc. of cream were injected into the duodenum. After ten minutes the animals were killed and the contents of the stomach and small intestines recovered and analyzed in the above manner.

These washings were evaporated to dryness in an oven at 80°C., and the dry weight of the residue was determined. After thorough mixing the lipid material from a weighed aliquot of the residue was extracted.

The procedures used for determining total and free cholesterol, lipid phosphorus, and total lipid carbon were, with some modifications, the manometric methods described by Kirk, Page and Van Slyke (9) and Van Slyke and Folch (17).

The lipids were extracted by 90 cc. of alcohol-ether at room temperature, which was found by Boyd (3) to be entirely satisfactory. Cholesterol esters were saponified by adding to the aliquot 0.18 cc. of a saturated aqueous solution of sodium hydroxide and 2 cc. of ethyl alcohol. Another modification was the substitution of the procedure of Man (11) for the evaporation of the alcohol-ether extract prior to extraction with petroleum ether.

The values obtained by these methods were converted into the more conventional terms by the calculations given by Page et al. (13), with the exception of true free cholesterol which was obtained by multiplying the observed free cholesterol by 0.8 (6, 16).

RESULTS. *Lymph:* A change in the concentration in lymph may be caused by a change in the quantity of the compounds reaching the lymph from the intestine, or by a change in the amount of fluid which reaches the lymph from the intestine or from contributory lymphatics. Since there are two variables which determine concentration of thoracic duct lymph, it is hazardous to draw conclusions from concentrations. We have accordingly used the expression quantity per hour rather than quantity per volume as a basis for comparison.

In table 1 are recorded the quantities of each lipid fraction for each period of collection. As would have been anticipated, the amount of total lipids and neutral fat in the lymph is greatly increased by intestinal absorption of lipids. In the two animals receiving milk, however, there was a decrease in the rate of total lipid and neutral fat transport.

In table 2 are found the data required for calculating the percentage of administered lipids that were absorbed and recovered in the lymph. We were able to recover an average of 93 per cent of the lipid injected into the duodenum in the control experiments, and the figures given in the third column have been corrected accordingly. In the case of dog 1 the absorp-

tion was not quite 70 per cent, while in the other two it was well above this. The figures in the fifth column are slightly too high, because the lipid which would have been present had no cream been given is included. Only a

TABLE 1

Lipid transport by lymph after injection of cream or milk

Results in milligrams

HOUR	SAMPLE	TOTAL CHOLESTEROL					FREE CHOLESTEROL					ESTERIFIED CHOLESTEROL				
		Cream			Milk		Cream			Milk		Cream			Milk	
		D1	D2	D3	D4	D5	D1	D2	D3	D4	D5	D1	D2	D3	D4	D5
0	L_0	22	5	11	6	78	5	1	4	2	21	17	4	7	4	57
0–1	L_1	25	6	13	6	55	6	2	2	2	16	19	4	11	4	39
1–2	L_2	17	16	9	*	80	4	5	2	*	25	13	11	7	*	55
2–4	L_3	28	33	11	6	54	9	14	5	2	16	19	19	6	4	38
4–6	L_4	21	33	14	4	43	7	16	7	1	12	14	17	7	3	31
6–8	L_5	18	35	6	7	36	6	13	4	3	11	12	22	2	4	25
8–10	L_6	18	27	11	6	25	6	10	1	2	7	12	17	10	4	18
10–12	L_7	22	38	2	5	14	7	12	0	2	5	15	26	2	3	9
12–14	L_8	21	35	1	5		7	9	0	2		14	26	1	3	
14–16	L_9	17	25		4		6	6		1		11	19		3	
16–18	L_{10}	17	21		4		6	5		2		11	16		2	
18–20	L_{11}	21	17		4		9	4		2		12	13		2	
20–22	L_{12}	12					5					7				
22–23	L_{13}	15					6					9				
		PHOSPHATIDE					NEUTRAL FAT					TOTAL LIPIDS				
0	L_0	14	3	4	12	27	45	4	16	9	38	92	15	37	29	182
0–1	L_1	20	2	0	10	61	78	4	10	13	45	136	16	30	32	187
1–2	L_2	12	15	0	*	63	46	24	11	*	95	83	62	25	*	277
2–4	L_3	25	46	18	10	60	104	224	127	11	75	170	317	160	30	215
4–6	L_4	21	71	7	7	43	161	399	262	10	59	224	517	287	24	166
6–8	L_5	25	61	0	10	24	166	199	157	13	43	219	310	164	34	120
8–10	L_6	23	32	0	8	22	147	99	15	13	20	196	169	32	30	79
10–12	L_7	30	39	0	9	13	186	52	13	9	19	248	148	17	25	54
12–14	L_8	29	23	0	9		182	28	2	10		242	104	4	26	
14–16	L_9	15	9		8		93	10		12		132	58		25	
16–18	L_{10}	12	4		9		75	7		9		111	44		24	
18–20	L_{11}	29	4		8		221	6		12		278	36		24	
20–22	L_{12}	17					126					158				
22–23	L_{13}	20					158					198				

* L_2 combined with L_3 and represents lymph flowing during 3 hours.

small fraction (4–17 per cent) of absorbed lipids is transported by the left thoracic duct lymph during and immediately after the period of absorption.

Instead of a greater percentage transportation of lipids by the left thoracic duct, as might have been expected if absorption were incomplete

in the experiments of Munk and Rosenstein (12) and Eckstein (5), our results were considerably lower than those of the former authors and very similar to those of Eckstein. This deviation from the results of Munk and Rosenstein might be explained by the fact that their fasting period was much shorter than ours; it must also be remembered that they were using as subject a person with a definite pathological condition—elephantiasis.

The control animals showed a decrease in lymph phosphatide which was completely or partly overcome when cream was given. This increase was not related to the small amount given (80–190 mgm.) but was related to the rate of lipid absorption. This may be construed as evidence that phosphatide is synthesized during absorption of lipid. In previous reports it has been assumed that an increase in the concentration of phosphatides in thoracic lymph is evidence of synthesis. For reasons given above, mere changes in concentration are unreliable indications of changes in quantity.

Changes in the cholesterol fractions of lymph were not significant.

TABLE 2

Per cent of absorbed lipids recovered in lymph

DOG NUMBER	LIPID INJECTED AS CREAM	LIPID RECOVERED FROM INTESTINE	LIPID ABSORBED	LIPID RECOVERED IN LYMPH	PER CENT OF ABSORBED LIPID RECOVERED
	mgm.	*mgm.*	*mgm.*	*mgm.*	
1	21065	6999	14066	2395	17
2	20659	564	20095	1781	9
3	17739	1641	16098	716	4

Plasma: To facilitate the analysis of the data obtained for plasma the percentage change from the concentration found in the samples drawn in the control period were calculated for each lipid fraction for each period and for each dog. These percentages were then averaged to obtain a composite for each lipid fraction in blood from each of the two sources and for each type of experiment—injection of cream or milk. These data are presented in tables 3 and 4.

It has generally been assumed that if the entrance of thoracic duct lymph into the blood is prevented by ligation or by cannulation of the duct, any absolute increase in lipid concentration in the portal blood, or any relative increase in the portal when compared with some other blood, is evidence of direct blood transport. This assumption is hazardous because in the first place it has been shown (2, 10) that ligation of the duct frequently results in the establishment of collateral lymphatic circulation with consequent entrance of lymph into the blood by other channels, and in the second place because no attempts have been made to determine what effect the digestion and absorption of non-lipid materials had upon the blood lipid concentration.

We have found definite increases in the concentration of total lipids, neutral fat, phosphatide and free cholesterol (with a corresponding decrease in the bound form) in both the portal and femoral plasmas during

TABLE 3

Average per cent changes in plasma concentration above and below zero hour concentrations after administration of cream

P = portal, F = femoral

HOUR	TOTAL CHOLESTEROL		FREE CHOLESTEROL		ESTERIFIED CHOLESTEROL		PHOSPHATIDE		NEUTRAL FAT		TOTAL LIPIDS	
	P	F	P	F	P	F	P	F	P	F	P	F
1	−6.0	−2.0	+3.0	+3.0	−11.0	−4.0	−4.0	−12.0	+28.0	−3.0	0	−7.5
2	−3.0	−2.0	−2.0	−0.5	−3.0	−3.0	+15.0	−7.0	+7.0	+5.0	+2.0	−5.0
4	0	−1.0	+12.0	+11.0	−5.0	−6.0	+31.0	0	+30.0	+28.0	+13.0	−1.0
6	+2.0	0	+18.0	+15.0	−6.0	−7.0	+28.0	+1.0	+34.0	+13.0	+14.0	+2.0
8	+3.0	+3.0	+19.0	+25.0	−4.0	−7.0	+23.0	−1.0	+35.0	+40.0	+14.0	+7.5
10	+10.0	+6.0	+22.0	+22.0	+4.0	−1.0	+19.0	+5.0	+34.0	+44.0	+16.0	+12.0
12	+3.0	+1.0	+30.0	+26.0	−7.0	−12.0	+22.0	+5.0	+40.0	+41.0	+13.0	+10.0
14	+9.0	+7.0	+24.0	+26.0	+2.5	−2.0	+30.0	+8.0	+30.0	+26.0	+17.0	+9.0
16	+4.0	−2.0	+21.0	+24.0	−3.0	−15.0	+17.0	−4.0	+17.0	+57.5	+8.0	+10.0
18	+8.0	0	+40.0	+24.0	−6.0	−9.0	+52.0	−18.0	+36.0	+35.0	+21.0	−2.0
20	−2.0	−4.0	+16.0	+14.0	−9.0	−11.0	−11.0	−15.0	+16.0	+22.0	−3.0	−7.0

TABLE 4

Average per cent changes in plasma concentrations above and below zero hour concentrations after administration of milk

P = portal, F = femoral

HOUR	TOTAL CHOLESTEROL		FREE CHOLESTEROL		ESTERIFIED CHOLESTEROL		PHOSPHATIDE		NEUTRAL FAT		TOTAL LIPIDS	
	P	F	P	F	P	F	P	F	P	F	P	F
1	−5.0	+1.5	+4.0	+2.0	−9.0	+1.5	−9.0	−1.5	+30.0	+7.0	−0.5	+1.5
2	−1.5	+5.0	−7.5	−3.5	+1.0	+10.0	+20.0	+10.0	−7.5	+8.0	+2.5	+7.5
4	+1.0	+6.0	+4.0	+6.0	0	+5.5	−11.0	−2.0	+57.0	+6.0	+6.0	+3.0
6	+1.0	+5.0	+3.5	+2.0	+0.5	+6.5	+25.0	−13.0	+126.0	+0.5	+32.0	−3.0
8	+5.0	+11.5	+11.5	0	+2.0	+17.0	+30.0	−21.0	+5.0	−14.0	+12.5	−6.5
10	+3.5	+0.5	+13.0	+4.0	0	−1.5	+39.0	−0.5	+2.0	+6.0	+13.5	+2.0
12	−0.5	+11.5	+14.0	+1.5	−8.5	+17.0	+29.0	−14.0	+25.0	−9.0	+10.0	−3.0
14	+1.5	+9.5	+20.5	+19.0	−7.0	+3.5	+23.0	+4.5	+61.0	+40.0	+14.5	+15.0
16	−3.0	+1.0	+33.5	+18.5	−22.0	−9.0	+10.0	+4.0	+52.5	+19.0	+12.0	+12.5
18	−9.5	+6.5	+11.5	+25.0	−28.5	−5.0	+22.0	−18.0	+9.0	+7.0	+10.5	−4.5

the absorption of lipids. In most cases the rate of increase was greater in the portal than in the femoral. If these were the only data available, one would conclude that lipids are transported directly by the portal vein during absorption. However, since almost identically the same increases

in portal lipid concentration were found in the control animals which were absorbing non-lipid material, it must be concluded that changes in portal lipid concentration are not necessarily related to the absorption of lipid *per se* and cannot be considered as evidence for the direct transportation of absorbed lipids.

The explanation may be that there is a mobilization of lipids from depots, perhaps in the intestinal wall or mesentery. The stimulus for this mobilization must be the absorption process itself quite independent of the specific absorption of lipids. It is unlikely that experimental procedures are responsible since Eckstein (4) found that experimental manipulation and anesthesia alone produced no lipemia in femoral blood. It is likely that the so-called alimentary lipemia is due to two factors: *a*, entrance into the general circulation of absorbed lipids which are transported by the lymphatic system, and *b*, the mobilization of lipids by the portal vein from depots located in its area of drainage.

On the other hand, there is evidence that neutral fat and phosphatides do reach the general circulation during lipid absorption. It is probable that this is due to the presence of lymphatico-venous communications other than the well recognized one between the thoracic duct and the neck veins. This is a factor which has been almost universally neglected in studies of lipid absorption. Schmidt-Mülheim (14), Baum (1), Silvester (15), and Job (8) have described such communications.

It is of some interest that in the case of neutral fat, and for that reason also in the case of total lipid, there is a marked and rapid increase in femoral plasma concentration which appears late in the experiment. This is probably due to a delayed mobilization of neutral fat in the general circulation and appears to be a terminal phenomenon. This would explain the rather prolonged elevation in plasma concentrations found in the animals absorbing lipid.

SUMMARY

In three dogs the percentage of absorbed lipids which was transported by the left thoracic duct lymph during and immediately after absorption of lipids varied from 4 to 17 per cent. The rate of transport of total lipids, neutral fat and phosphatides increased during absorption.

There is a greater increase in the proportion of cholesterol in the free state in the general circulation during the absorption of lipids than during the absorption of non-lipids. There is a definite increase in the neutral fat concentration in the general circulation during the absorption of lipids and little change during the absorption of non-lipid material. There is little change in the concentration of phosphatide in the general circulation during the absorption of lipids, but there is a marked decrease during the absorption of non-lipids. These results are evidence that neutral fat and

phosphatide enter the general circulation during lipid absorption, probably by way of lymphatico-venous communications.

There is no evidence that absorbed lipid enters the portal blood directly. The increase in portal plasma lipids during non-lipid, as well as lipid absorption, suggests a mobilization of stored lipids by the portal blood under the stimulus of absorption itself. This phenomenon is being investigated further.

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THE EFFECT OF ACIDOSIS UPON THE RENAL TUBULAR REABSORPTION OF PHOSPHATE

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In a previous report (1) we demonstrated that the rate of reabsorption of phosphate by the renal tubules is an important factor in the regulation of the concentration of phosphate in the blood plasma at equilibrium. Vitamin D was found to increase the rate of renal tubular reabsorption of phosphate, thereby increasing the concentration of this ion in the plasma and other extracellular fluids. On the other hand, parathyroid extract decreased the rate of reabsorption of phosphate by the renal tubules and lowered the concentration of phosphate in the plasma. It has been repeatedly observed that the excretion of phosphate in the urine is increased in states of acidosis (2). Following the administration of acidifying salts to animals or man the increase in urine phosphate is not associated with an increased concentration of phosphate in the plasma but rather with a decrease in the plasma phosphate. In recent years cases of rickets not due to vitamin D deficiency have been reported in which a low concentration of phosphate in the serum was found associated with a severe chronic acidosis (4, 5, 6). It seemed probable that the rate of reabsorption of phosphate by the renal tubules was reduced in states of acidosis. The present experiments were designed to test this hypothesis.

The experiments were performed on dogs in whom an acidosis was produced by the oral administration of ammonium chloride. The degree of acidosis was measured by the reduction in serum bicarbonate as well as by determination of plasma pH. The rate of tubular reabsorption of phosphate was estimated by simultaneous determinations of the plasma creatinine and phosphate clearances following the intravenous injection of sodium phosphate. Since, in the dog, the plasma creatinine clearance is a measure of the filtration rate the difference between the creatinine and phosphate clearances can be used to calculate the amount of phosphate which has been reabsorbed by the renal tubules. The detailed procedure has been previously described (1).

When ammonium chloride is given in sufficiently large amounts to produce an acidosis a considerable loss of extracellular fluid volume and hemo-

concentration may result and consequently a reduction in the rate of glomerular filtration as measured by the creatinine clearance is found. It was therefore first necessary to study the effect of variations in the rate of glomerular filtration upon the tubular reabsorption of phosphate. In many of the experiments in which spontaneous fluctuations in the rate of glomerular filtration occurred during successive periods, the rate of tubular reabsorption appeared to vary with the filtration rate. These findings were not entirely constant and the effect of marked variations in the rate of glomerular filtration was therefore determined. This was accomplished by decreasing or increasing the extracellular fluid volume sufficiently to produce a significant decrease or increase in the filtration rate. A de-

TABLE 1

Effect of dehydration upon glomerular filtration and renal tubular reabsorption of phosphate

TREATMENT	URINE VOLUME	CREATININE CLEARANCE	PHOSPHATE REABSORBED	
			Mgm. per min.	Mgm. per 100 cc. glom. filtrate
Dog B				
	cc. per min.	*cc. per min.*		
Control	1.3	50.5	2.24	4.4
5 per cent glucose I.P.	1.2	34.5	1.47	4.3
Dog A				
Control	1.4	32.8	1.04	3.2
Control	1.8	33.1	1.07	3.2
Salyrgan	2.1	27.1	0.93	3.4
5 per cent glucose I.P.	0.5	8.8	0.36	4.1
Control	1.8	33.3	1.18	3.5

creased extracellular fluid volume was produced by the intraperitoneal injection of 5 per cent glucose and removal 5 hours later of the peritoneal fluid according to the technique of Darrow and Yannet (7). A mild degree of dehydration was also produced in one animal by the injection of a mercurial diuretic, salyrgan. Increase of the filtration rate was brought about by the continuous intravenous injection of a solution of sodium chloride and sodium bicarbonate of approximately the composition of extracellular fluid. The rate of injection was varied from 5 to 10 cc. per minute for a period of 2 hours.

The results of these experiments are shown in tables 1 and 2. In the experiments in which dehydration had been produced sufficient water was given by stomach tube at the time of the experiment to maintain an ade-

quate flow of urine. The rate of reabsorption of phosphate is expressed both as milligrams phosphorus per minute and as milligrams phosphorus per 100 cc. of glomerular filtrate. When the rate of glomerular filtration, as measured by the creatinine clearance, is reduced there is seen to be an approximately proportionate reduction in the rate of reabsorption of phosphate by the renal tubules. The amount of phosphate reabsorbed in terms of volume of glomerular filtrate therefore remains fairly constant. However, when the rate of filtration is increased above the normal by a continuous intravenous injection, the tubular reabsorption of phosphate reaches a maximum which is not increased by further increase in the filtration rate. The equilibrium concentration of phosphate, i.e., the concentration of phosphate in the plasma at which the amount filtered through the glomeruli equals the amount reabsorbed by the renal tubules is therefore reduced by increasing the rate of glomerular filtration. Shannon and Fisher (8) have shown that in the normal dog the tubular reabsorption of glucose reaches a maximum which remains constant despite changes in the rate of filtration.

TABLE 2

The effect of increased glomerular filtration upon rate of renal tubular reabsorption of phosphate

CREATININE CLEARANCE	URINE VOLUME	PHOSPHATE REABSORBED	
		Mgm. per min.	Mgm. per 100 cc. glom. filtrate
	Dog C		
cc. per min.	*cc. per min.*		
62.3	0.5	3.39	5.44
65.5	0.6	3.51	5.36
Constant intravenous injection of saline bicarbonate solution			
71.3	0.6	3.54	4.97
75.3	0.4	3.68	4.89
81.2	1.2	3.74	4.61
88.5	1.9	3.76	4.25
	Dog D		
41.6	0.4	1.24	2.98
39.3	0.3	1.14	2.90
Constant intravenous injection of saline bicarbonate solution			
42.0	0.4	1.20	2.86
44.9	0.6	1.11	2.47
48.4	1.3	1.09	2.25
53.4	2.5	1.12	2.10
57.7	4.6	1.10	1.96

In their experiments a constant intravenous infusion of glucose solution was given and the conditions are therefore comparable to those experiments in which saline was injected. In the dehydrated dog the decrease in the rate of tubular reabsorption of phosphate in proportion to the decrease in filtration rate suggests that many of the glomeruli are not functioning and therefore no phosphate is available for reabsorption by the tubules connected with them. However, this phenomenon of glomerular intermittence which has been observed in the amphibian (9) has not been demonstrated in the normal dog by the injection technique (10). No studies by this method have been made in the dehydrated dog. Whatever the explanation may be it is apparent that in states of dehydration the reduction in filtration rate is associated with a comparable reduction in the estimated rate of phosphate reabsorption. For purposes of comparison it is preferable to express the reabsorption of phosphate in these experiments in terms of milligrams phosphorus per 100 cc. of glomerular filtrate.

TABLE 3

The effect of acidosis on renal tubular reabsorption of phosphate

Dog A

DATE	TREATMENT	CREATININE CLEARANCE	PHOSPHATE REABSORBED		SERUM	
			Mgm. per min.	Mgm. per 100 cc. glom. filtrate	P	CO_2
		cc. per min.			*Mgm. per 100 cc.*	*volume per cent*
12/29	Control	30.8	1.28	4.2	5.6	50.0
1/2–1/4	NH_4Cl—5 grams daily					
1/5		24.8	0.90	3.7	3.2	29.0
1/8	Recovery				4.0	50.7
1/21–1/23	NH_4Cl—7 grams daily					
1/24		26.5	0.69	2.6	3.0	16.6
2/27	Control	29.9	1.25	4.2	4.6	
3/2–3/4	NH_4Cl—5 grams daily					
3/5		29.3	0.95	3.2	4.1	30.4

The results of the experiments in which ammonium chloride was given are shown in tables 3, 4 and 5. Control studies were done both before and after the acidosis experiments and the experimental prodecure was identical in all cases. The plasma pH was determined in many of the experiments, using a Leeds and Northrup Universal pH indicator employing a glass electrode which permitted the determination without exposure of the sample to air. A reduction of plasma pH was found consistently following the administration of ammonium chloride in the amounts given and the reduc-

TABLE 4

The effect of acidosis on renal tubular reabsorption of phosphate

Dog B

DATE	TREATMENT	CREATININE CLEARANCE	PHOSPHATE REABSORBED		SERUM	
			Mgm. per min.	Mgm. per 100 cc. glom. filtrate	P	CO_2
		cc. per min.			*Mgm. per 100 cc.*	*volume per cent*
12/3	Control	57.2	3.73	6.5	7.2	
12/6–12/7	NH_4Cl—10 grams					
12/8	NH_4Cl—5 grams					
12/9	Vomited NH_4Cl given					
12/10		47.2	2.22	4.7	4.6	38.7
12/17	Recovery	50.8	3.05	6.0	6.5	60.0
1/2–1/6	NH_4Cl—5 grams daily					
1/7		42.8	2.51	5.8	6.4	45.0
1/12–1/15	NH_4Cl—10 grams daily					
1/16		47.0	2.28	4.9	5.1	36.2
1/21	Recovery	45.6	2.57	5.6	6.4	62.4

TABLE 5

The effect of acidosis on renal tubular reabsorption of phosphate

Dog C

DATE	TREATMENT	CREATININE CLEARANCE	PHOSPHATE REABSORBED		SERUM	
			Mgm. per min.	Mgm. per 100 cc. glom. filtrate	P	CO_2
		cc. per min.			*Mgm. per 100 cc.*	*volume per cent*
4/10	Control	51.8	3.63	7.0	7.3	47.2
4/15	NH_4Cl—10 grams					
4/16	NH_4Cl—7.5 grams					
4/17		47.8	2.34	4.9	5.1	19.2
4/18	Recovery				6.3	38.1
4/22	Recovery	56.7	3.32	5.9	6.1	48.2
5/13	Control	63.9	3.45	5.4	5.5	
5/23–5/29	NH_4Cl—6 grams daily					
5/29		60.7	2.54	4.2	4.0	28.5
5/30–6/3	NH_4Cl—6 grams daily					
6/2					3.1	21.3
6/3		43.4	1.43	3.3	2.8	28.0
6/11	Recovery	67.7	2.20	3.3	3.9	46.7
6/17	Recovery	66.6	2.90	4.4	5.2	46.6

tion ranged from 0.11 to 0.27 pH unit. The degree of reduction in pH paralleled the decrease in serum bicarbonate.

In the tables are listed the plasma creatinine clearance and the calculated rate of tubular reabsorption of phosphate expressed both as milligrams phosphorus per minute and as milligrams phosphorus per 100 cc. of glomerular filtrate. The concentration of phosphate and of bicarbonate in the serum in the fasting state on the morning of the experiment is also given.

There is obviously a considerable decrease in the rate of tubular reabsorption of phosphate following the development of a severe acidosis. In those experiments in which the creatinine clearance is lowered the reduction in the rate of tubular reabsorption of phosphate is far greater than that expected as the result of the diminished filtration rate. This is shown by the fact that the reabsorption per volume of glomerular filtrate is decreased. The magnitude of the decrease in each animal is to some extent proportional to the severity of the acidosis. This is shown particularly by the results for dogs A and B in tables 4 and 5, respectively. The results for dog B are of particular interest. On 1/7 despite the administration of 5 grams NH_4Cl for 4 days there is only a mild degree of acidosis and no significant reduction in the rate of tubular reabsorption of phosphate is found. The administration of NH_4Cl was discontinued and the animal permitted to recover. A second course of NH_4Cl treatment was followed by a more severe acidosis. At this time 1/16, a definite decrease in the rate of renal tubular reabsorption of phosphate, is found.

The decrease in tubular reabsorption of phosphate appears to be related not only to the severity of the acidosis but also to the duration. In the experiments on dog C a moderate degree of acidosis was produced on 5/29 and the rate of tubular reabsorption of phosphate determined. The administration of NH_4Cl was continued in amounts sufficient to keep the serum bicarbonate at the same low level and it is seen that after a total of 11 days of NH_4Cl treatment there is a much greater decrease in the rate of tubular reabsorption which is not due merely to the greater reduction in the filtration rate.

The recovery of renal tubular function following cessation of the NH_4Cl administration is also an interesting phenomenon. In table 3 the results of the experiment on dog A indicate a complete return to the control values following the cessation of ammonium chloride treatment. This was an adult animal in whom the rate of tubular reabsorption of phosphate is fairly constant under normal conditions. In the case of dogs B and C the rate of tubular reabsorption of phosphate in the recovery experiments does not return completely to the preacidosis level. This probably is explained by the fact that these were animals less than 18 months of age and at this period a gradual decrease in the rate of tubular reabsorption of phosphate is normally observed (1). The recovery process is seen to be a rapid one

in those experiments in which an acidosis of short duration was produced. However, in the experiment on dog C shown in table 5 in which the acidosis was more prolonged it may be seen that the rate of tubular reabsorption of phosphate remained low for at least one week following cessation of NH_4Cl treatment even though the serum bicarbonate returned to normal. After 14 days, however, a definite increase in the rate of tubular reabsorption of phosphate is found although still below the control level. In another experiment on dog B there was also suggestive evidence that the renal tubular reabsorption of phosphate may return only slowly to the normal level even though the normal acid base equilibrium of the plasma has been restored. In this experiment the concentration of plasma phosphate remained low for several days after the bicarbonate had been restored to normal.

The concentration of phosphate in the plasma in the postabsorptive state is seen to parallel closely the rate of tubular reabsorption of phosphate as expressed in milligrams phosphorus per 100 cc. of glomerular filtrate. This is in accord with the previously expressed idea that the concentration of phosphate in the plasma tends to approach the "equilibrium concentration", i.e., the concentration at which the rate of filtration through the glomeruli equals the rate of reabsorption by the renal tubules (1). Numerically this equilibrium concentration is identical with the value of the phosphate reabsorbed per 100 cc. of glomerular filtrate.

The serum calcium was determined in all of the experiments. No consistent change in the concentration of serum calcium was noted in these studies.

Discussion. The effect of acidifying salts upon the renal tubular reabsorption of phosphate is similar to that of parathyroid extract (1). Both cause a decrease in the rate of renal tubular reabsorption of phosphate and therefore a decreased concentration of phosphate in the plasma. The effect of acidosis differs from that due to hyperparathyroidism in that there is no concomitant elevation of the concentration of calcium in the serum. The results of the present experiments offer an explanation for the low concentration of phosphorus in the serum which may be found in states of chronic acidosis. The exact mechanism by which the acidosis affects the function of the renal tubules is still unknown.

The loss of phosphate from the body following the administration of acidifying salts is in excess of that which could come from the extracellular fluids of the body alone (3). There is direct evidence that this excess is derived from the intracellular organic phosphates since the acid soluble phosphate of the red blood cells is found to be decreased in states of acidosis (3,11). It is quite probable that there is an interrelationship between the effect of acidosis on renal tubular reabsorption of phosphate and the decrease of intracellular phosphate. The present study emphasizes the

fundamental importance of renal tubular function in maintaining the concentration of phosphate in the plasma and other body fluids at normal levels.

SUMMARY

The rate of reabsorption of phosphate by the renal tubules was determined in dogs in whom an acidosis was produced by the administration of ammonium chloride. It was found that following the development of an acidosis there was a decrease in the rate of tubular reabsorption of phosphate which was related both to the severity of the acidosis and its duration. The decreased concentration of phosphate in the plasma following the administration of acidifying salts can be explained by this effect on the renal tubular reabsorption of phosphate.

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THE PRESSOR RESPONSE OF NORMAL AND HYPERTENSIVE DOGS TO RENIN AND ANGIOTONIN

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Inconclusive evidence suggests that the blood vessels of hypertensive animals and man are abnormally sensitive to pressor agents, hence no more than normal amounts of them in the blood would be required to cause hypertension. It is, therefore, of importance to learn whether this is true of such substances as renin and angiotonin because of the belief that they may be involved in the genesis of chronic arterial hypertension. It is this problem with which this investigation is concerned.

Purified renin is not of itself a pressor agent and requires the intervention of renin-activator (1) before eliciting vasoconstriction by formation of angiotonin (2, 2a). In normal animals the supply of renin-activator is easily exhausted, but more of this substance appears available in the blood of hypertensive animals (3). It would, therefore, be anticipated that hypertensive animals would respond to injections of renin with a greater or, at least, more prolonged rise of arterial pressure than normal animals simply because more angiotonin is formed rather than that hypertensive animals are more sensitive to the action of angiotonin; hence increased pressor sensitivity of hypertensive animals might reflect an increased supply of renin-activator. An altered response of the vessels themselves to renal pressor agents can only be assumed if it can be shown to exist with reference to the effective pressor substance, angiotonin.

METHODS. Dogs of from 10 to 14 kgm. weight were anesthetized by intraperitoneal injections of 30 mgm. of pentobarbital per kilogram body weight. The femoral artery was cannulated and connected to a mercury manometer by a tube filled with heparin solution. Injections were made into the femoral vein.

Renin was prepared by the method of Helmer and Page (4), and angiotonin by that of Page and Helmer (5). Single injections of renin always consisted of 0.3 cc., and angiotonin 0.25 cc., the angiotonin being given before the renin. When renin was infused, it was diluted in the proportion of one part in ten of physiological saline solution and given at the rate of 0.8 cc. per minute. The infusion was discontinued after 45 minutes. The

animal often was retested a week later and then hypertension induced by wrapping one or both kidneys in silk (6). Usually within three weeks or more, hypertension of the order of 180 to 210 mm. Hg mean pressure had developed and the tests were repeated. Blood pressure was measured in these animals usually on alternate days by femoral intra-arterial puncture with a no. 20 needle.

RESULTS. It was soon evident that the pressor response of the different normotensive dogs varied greatly, especially to renin and less so to angiotonin. The same animal tested on different occasions exhibited less variability. At first we thought this might be due to different depths of anesthesia. This is apparently not the case for, in 6 animals, increasing the dose of pentobarbital progressively had no regular effect on the pressor response to angiotonin. In several docile, well-trained animals tests were made without anesthesia and later compared with those made while the animal was anesthetized. Just as had been found before (7), no regular differences were encountered; however, since some of the animals became excited, especially at the height of the blood pressure rise, it was believed that experimentally comparable conditions could best be attained by the use of pentobarbital anesthesia. Furthermore, few dogs could be trained to lie quietly for 40 minutes during an infusion of renin.

It also seemed desirable to determine whether the level of blood pressure could consistently influence the results. Experience shows that at both extremes of blood pressure the response to *angiotonin* is reduced. When arterial pressure is elevated or depressed less extremely, there does not appear to be any direct relationship between the level of blood pressure and the pressor response. For example, the responses to angiotonin of one dog with arterial pressure at 240 mm. Hg were 18 and 14 mm. Hg, but one with arterial pressure of 174 also showed similar responses, namely, 20 and 14 mm. Hg. One dog with arterial pressure at 224 mm. Hg responded to angiotonin by rises of 36 and 32 mm. Hg while in another with blood pressure of 180 the responses were also 36 and 34 mm. Hg.

Page (7) was unable to demonstrate any deciding influence on the pressor response to *renin* of the initial level of blood pressure in normal animals. As table 1 shows, the same appears to be true of hypertensive animals. It makes no difference whether the initial blood pressure chosen is the *average* daily pressure determined without anesthesia or one measured under anesthesia, just before the renin is administered. For example, a rise of 48 mm. Hg occurred in an animal in which the average daily pressure was 224 mm. Hg and the initial pressure under anesthesia was 162 mm. Hg. Yet a rise of 50 mm. Hg occurred in another animal with an average daily pressure of 228 mm. Hg, but an initial pressure under anesthesia of 210 mm. Hg. It is possible that at extremes of pressure, such as 230 mm. Hg and 240 mm. Hg mean pressure, the response is reduced.

Having selected suitable conditions for testing the response of the animals to angiotonin and renin, 6 dogs were tested; in two cases (no. 13 and no. 8) twice, and hypertension induced by silk perinephritis. When various levels of elevation of mean arterial pressure ranging from 140 to 224 mm. Hg were attained, the animals were again tested (table 2). While there was a slight increase in the response to angiotonin it was not consistent. The response to renin was slightly greater in all experiments.

It seemed desirable to ascertain the sensitivity in a different way. Instead of giving the renin by single injections, it was infused in dilute solution. Six animals were tested in this manner before and after the induction of hypertension (table 3). Again the same range of blood pressure levels

TABLE 1

Relationship of renin pressor response to arterial blood pressure in hypertensive dogs

DOG NUMBER	AVERAGE B.P. LEVEL OF UNANESTHETIZED DOG	B.P. BEFORE INJECTION, WHILE ANESTHETIZED	RISE AFTER RENIN
			mm. Hg.
1	174	190	22
2	180	210	22
3	180	160	28
4	180	184	28
5	182	214	34
6	194	170	26
7	198	204	30
8	202	226	28
9	210	200	50
10	212	186	54
11	212	210	20
12	220	220	22
13	224	162	48
14	228	210	50
15	230	230	20
16	240	240	16

was chosen. The response to angiotonin, just as in the last group of experiments, was slightly greater but not consistently greater in the hypertensive animals. With one exception (no. 20), the response to infused renin was both greater and more prolonged even on repeated infusions. These experiments thus support the results of the group in which the increased response of hypertensive animals to renin was shown by single injections.

It then seemed desirable to test a larger series of normal and hypertensive dogs with single injections of both angiotonin and renin, but in this group the same animals were not necessarily tested before and after the induction of hypertension. Amounts of each were used such that the rise in blood pressure was not excessive. As Swingle, Taylor, Collings and Hays (8)

have shown, when small doses of renin are employed the initial blood pressure does not effect the pressor response elicited. Fifty-seven normotensive animals were tested with angiotonin and thirty-six hypertensive, the blood pressure of the latter all being over 170 and averaging 200 mm. Hg.

It was necessary to use such a large number of animals because the variability in response from one animal to another was great. In table 4 the normal and hypertensive animals are grouped together according to the particular sample of angiotonin used for test purposes. The results show

TABLE 2

Pressor response of dogs to renin and angiotonin before and after induction of perinephric hypertension

NORMAL DOGS				HYPERTENSIVE DOGS		
Dog number	Initial B.P.	Rise of B.P. after angiotonin	Rise of B.P. after renin	Average B.P. after induction of hypertension	Rise of B.P. after angiotonin	Rise of B.P. after renin
	mm. Hg					
17	122	14	22	140	24 24	28
16	134	10 12	16	160	28 30	28
18	130	30 28	10	174	20 14	22
19	120		20	198	30 18	30
8	116	26	20 20	202	22 18	28
13	138 144	22 24	32 32	224	36 32	48

that there is no significant difference in the response to angiotonin in normo- and hypertensive dogs.

Twenty-four normo- and hypertensive animals were tested with single injections of renin. The results on inspection show a small but consistent increase in sensitivity, which, however, is not statistically significant.

Discussion. The evidence concerning the sensitivity of the vascular system to pressor drugs in hypertension is conflicting. For this reason it is necessary to consider in some detail certain questions concerning the technique of the measurements of sensitivity and their interpretation.

Many of the conflicting reports are due in no small part to the inadequate

TABLE 3

Pressor response to single injections of angiotonin and infusions of renin before and after induction of hypertension

DOG NUMBER	INITIAL B.P.	RISE OF B.P. AFTER ANGIOTONIN	RESPONSE TO RENIN INFUSION				
			Under anesthesia				
			Initial B.P.	10	20	30	40 minutes
Before induction of hypertension							
	mm. Hg.						
20	132	18 14	172	18	18	4	0
21	122	18 20	136	32	40	34	24
22	130	14 14	204	16	0	−14	−22
23	120	16 14	180	14	6	2	0
24	134	20 22	148	42	32	20	16
25	118	28 20	164	36	36	14	−4
After induction of hypertension							
	AVERAGE B.P. AFTER HYPERTENSION						
20	180	8 10	266	6	−16	−36	−56
20	160	8 10	246	12	−22	−32	−40
21	176	16 16	190	44	44	38	26
21		16 16	182	36	28	12	8*
22	196	18 20	220	44	30	12	2
22	198	22 20	180	40	54	36	26
23	200	28 22	148	52	66	58	52
24	210	24 22	192	54	30	22	32†
25	192	36 34	196	48	44	44	30

* Malignant.

† Early malignant.

number of animals employed in the experiments. Experience shows that the variation from one animal to another is great, and even in the same animal sufficient changes in sensitivity occur to make exact comparison difficult. Experiments in which the same animal is tested before and after induction of hypertension therefore seem most reliable. The cause of this great variability is not known and deserves further study.

The use of pentobarbital anesthesia appears to be the lesser of two evils because it has been shown that in moderate amounts it does not greatly alter the intensity of the pressor response to renin and angiotonin, and even varying the depth of the anesthesia does not consistently alter the response one way or another.

The problem whether the response to pressor agents in general is directly dependent on the height of the arterial blood pressure is no simple one, even

TABLE 4

*Pressor response to single doses of angiotonin and renin compared in normal and hypertensive dogs**

LOT NUMBER	MEAN ± S.E. IN NORMAL DOGS	MEAN ± S.E. IN HYPERTENSIVE DOGS	VALUE OF t	D/F	VALUE OF t NECESSARY TO SHOW SIGNIFICANCE		SIGNIFICANT
					19/20	99/100	
Angiotonin 647	19.38 ±1.144	29.00 ±0.577	7.51	15	2.13	2.95	Definitely
Angiotonin 751	16.04 ±0.779	23.50 ±2.875	2.50	38	2.02	2.70	Barely
Angiotonin 226	26.95 ±2.325	22.67 ±2.658	1.21	50	2.01	2.68	No
Angiotonin 923	18.86 ±2.342	29.43 ±2.296	3.22	12	2.18	3.06	Yes
Renin 725	23.46 ±2.075	28.12 ±3.009	1.27	42	2.02	2.70	No

* We are grateful to Mr. E. B. Robbins and Dr. K. K. Chen for the statistical analysis.

when adrenalin is employed. The size of the dose, the rate of administration, the temperature of the animal and the intactness of the nervous system are all factors known to influence the response (9). When the blood pressure is lowered it depends on the means employed to lower it (10), and when adrenalin is injected repeatedly, the response depends on the number of injections (11–13).

The response to renin and angiotonin is not directly related to the initial height of the blood pressure. Immediately after pithing, it may be completely abolished and that to angiotonin greatly reduced. After several injections of angiotonin the response increases. Severe hemorrhage reduces the response, especially when the arterial pressure is markedly lowered. The method employed to lower pressure seems to determine whether an increased or decreased response will occur.

Some light is thrown by the results in table 1 on the question whether

arterial pressure measurements taken while animals are anesthetized correspond with those taken without anesthesia. It is clear that the variations of the blood pressure taken under pentobarbital anesthesia from the usual daily pressure taken by femoral arterial puncture without anesthesia is great in many cases. It is true that the pressures often correspond but not with sufficient regularity to justify use of the method except where large groups of animals are employed.

We are now in a position to correlate the results of experiments in which the purpose was to ascertain whether hypertensive animals respond more actively to angiotonin and other pressor agents than do normotensives.

Verney and Vogt (14) studied the pressor response of 7 hypertensive dogs to adrenalin. In one a decreased response was observed, in 3 no change, and in 3 others an increase was noted.

Appelrot (15) claimed that 10 dogs with hypertension produced by feeding large doses of vitamin D exhibited decreased rather than increased pressor responses to adrenalin when compared with normotensive dogs. Since blood pressure measurements were made under ether anesthesia without controls in which no anesthesia was employed, and since the hypertension was for the most part very moderate (average rise of 32 mm. Hg in 10 dogs fed vitamin D, compared with a 9 mm. Hg rise in 6 normal dogs), it is doubtful how much weight can be given these experiments.

The same irregularity of response was observed by Verney and Vogt (14), when tyramine was injected in single doses. The range of rise in normal dogs was 26 to 85, and hypertensive dogs 50 to 98 mm. Hg. When tyramine was infused into 3 dogs the hypertensives responded much *less* actively than normal ones. These results, obtained on a small number of animals, suggest that the vascular system of hypertensive animals is not hypersensitive to tyramine. Verney and Vogt were also unable to confirm Bouckaert, Elaut and Heymans' (16) observation that the pressor response to carotid sinus stimulations of 3 hypertensive dogs under chloralosane anesthesia, was increased.

Brown and MacGraith (17, 18) measured the response to drugs of 12 rabbits made hypertensive by constricting the renal artery, and 4 with glomerulonephritis induced by nephrotoxic serum. Blood pressure measurements were made on Van Leersum loops while the animals were under pentobarbital anesthesia. The degree of hypertension was not great. The pressor response to adrenalin, tyramine and posterior pituitary extract was increased and the depressor response to acetylcholine decreased as early as 10 hours after operation. Once the hypertension was established, the response varied roughly as the blood pressure level. In a later study (19) it was sought to determine whether the possible increase of sensitivity to tyramine was due to loss of tyraminase activity, but it was found that

slices of liver of hypertensive rats oxidized tyramine at normal rates. Ogden, Brown and E. W. Page (20) independently observed increased sensitivity in 16 of 17 rabbits in the pre-hypertensive stage, i.e., after constricting the renal artery but before any appreciable rise in arterial pressure had occurred. Similar studies have not been made in dogs.

Several studies have been carried out in which renin has been the pressor agent. While the renin employed in most cases leaves much to be desired from the point of view of chemical purity, nevertheless the results show a fair degree of consistency. Thus Leiter and Eichelberger (21) state in an abstract of their work that 20 injections into 4 unanesthetized hypertensive dogs caused a more sustained rise of blood pressure than in control animals. Page and Helmer (5) compared the response of 9 normal and 2 hypertensive dogs to infusions of renin. The hypertensive dogs showed both a greater and more sustained rise in pressure. Katz and Friedberg's (23) results with single injections of renin show slightly greater pressor response to renin in 3 of 4 hypertensive dogs, but they interpret these results as indicating similar responses in normo- and hypertensive dogs.

Heated kidney extracts tested by Kapp, Friedland and Landis (24) caused a greater rise of arterial pressure in 3 unanesthetized hypertensive rabbits than in 3 normal ones. When more concentrated extracts were used and the initial blood pressure was higher, the results in 5 hypertensive rabbits became irregular. According to Williams, Wegria and Harrison (25), 4 hydronephrotic rats with hypertension showed an average rise of about 20 mm. Hg greater than 3 control rats when renin was injected.

The more extensive data reported in this paper makes it appear probable that hypertensive dogs are slightly more sensitive than normal to renin, especially when subjected to the continuous administration of renin. This we interpreted as being due to increased renin-activator content of the blood of hypertensive animals (3), with formation of greater amounts of angiotonin. To angiotonin itself there seems to be no greater than normal pressor response in hypertensive dogs.

These experiments lend no support to the belief that the vessels of hypertensives are abnormally sensitive to substances which may participate in the chemical mediation of hypertension. Since angiotonin and renin may be among these substances, this observation assumes some importance.

SUMMARY. 1. The pressor response to renin and angiotonin is not consistently altered by moderate pentobarbital anesthesia. The initial arterial pressure level of hypertensive dogs may be profoundly altered by anesthesia.

2. The pressor response of different animals varies widely, but that of the same animal is relatively constant even at different times.

3. The pressor response to angiotonin and renin is not directly related to the initial arterial pressure. The means employed to lower or raise the pressure appear to determine in large measure the response.

4. Induction of hypertension in dogs does not alter the pressor response to angiotonin but increases the response to renin slightly, especially if the renin is administered by infusion.

CONCLUSION

Induction of experimental renal hypertension in dogs does not increase the pressor response to angiotonin. On the other hand, in such animals renin causes somewhat greater responses. The increased response to renin appears to be due to greater formation of angiotonin from combination of renin and renin-activator, and is not the result of increased sensitivity of the vascular system of hypertensive animals.

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THE EFFECT OF INSULIN ON CARDIAC AND LIVER GLYCOGEN

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Earlier observations (1) using subcutaneously injected insulin in rats fed glucose by mouth gave irregular effects on cardiac glycogen even though the deposition of gastrocnemius glycogen was marked in each animal. When the results for animals showing hypoglycemic signs and low terminal blood sugars were separated from the others, it appeared that hypoglycemia was accompanied by a lowering of cardiac glycogen, whereas in the animals in which hypoglycemia apparently did not occur, the cardiac glycogen was raised as compared either to 24 hour fasted controls or to such controls given glucose by mouth. To clarify these irregular results, the observations recorded in this paper, and employing intravenously injected glucose and insulin, were made.

The action of insulin on liver glycogen has been difficult to reconcile with its other known actions (2, 3, 4, 5). The marked raising of the low liver glycogen of diabetic animals by insulin injection seemed to be a potent argument in favor of its effect being like that seen for muscle glycogen; but it has been recognized for normal animals that insulin given with glucose resulted in a lower liver glycogen than would have occurred if glucose alone had been given. Because the experimental arrangements for observing cardiac glycogen could also conveniently include observations on liver, the two were made together, it being felt for reasons which will be mentioned in discussion that the insulin effect on liver glycogen could stand re-observation.

EXPERIMENTAL. Male rats, 24-hour fasted, weighing between 150 to 200 grams were lightly anesthetized with nembutal and given glucose by constant intravenous injection at the rate of 500 mgm. per 100 grams of rat per hour; to this injected fluid in some of the experiments insulin was added. The urine was collected and analyzed for glucose. At the termination of the injection period, a gastrocnemius, the heart, liver and a sample of blood were obtained. Analyses were carried out as previously described (1, 6, 7). The adrenalectomized animals were maintained on 1 per cent sodium chloride solution as drinking water and used on the third day postoperatively. The results are given in the table.

DISCUSSION. *Heart.* Intravenous glucose alone effected a definite

increase of cardiac glycogen. This was rather surprising in view of the earlier observation that glucose by mouth did not raise cardiac glycogen; indeed, the cardiac glycogen both of normally fed animals and also of 24-hour fasted animals absorbing glucose maximally for 4 hours was significantly lower than that of 24 or 48-hour fasted animals (1). In the present experiments the insulin is seen not to effect any further increase in cardiac glycogen. It would appear, therefore, either that the animals' own insulin acts maximally or that insulin is not necessary for the deposi-

TABLE 1

Effect of glucose and insulin intravenously

	GROUP	NUMBER OF ANIMALS	HOURS INJECTED	PER 100 GRAMS BODY WEIGHT			FINAL CONCENTRATION—CARBOHYDRATE MGM. PER CENT				MGM./100 BODY WEIGHT			
				Injected		Retained	Glycogen			Glucose	Increase in Glycogen			Carbohydrate not accounted for
				Glucose	Insulin units	Glucose	Gastrocnemius	Heart	Liver	Blood	Liver	Other	Total	
				mgm.		*mgm.*								
Intact	1	16					521 ±8*	542 ±17	192 ±32	87 ±5				
	2	13	2	500		880	750 ±14	657 ±23	1761 ±141	329 ±14	78 ±7†	114 ±11	192	567
	3	4	2	500	¼	941	877	648	1038	222	42	178	220	654
	4	4	2	500	½	959	846	687	824	204	31	162	193	708
	5	11	2	500	1	946	884 ±26	628 ±19	277 ±56	252 ±16	4 ±3	181 ±14	185	679
	6	5	2	500	2–5	974	900	694	141	434	−3	186	183	615
	7	4	2	500		1440	980	646	472	383	14	229	243	(1049)
			+1	500	1									
Adrex	8	12					462 ±13	387 ±41	136 ±8	58 ±4				
	9	4	2	500		904	553	428	1174	466	52	46	98	602
	10	4	2	500	1	936	690	457	159	382	1	114	115	659

* Standard deviation of mean.

† Standard error of difference.

tion of cardiac glycogen, this last interpretation being supported by the phenomenally high cardiac glycogen of diabetic animals (8), which in the light of these experiments may not be due simply to hyperglycemia.

The cardiac glycogen of adrenalectomized animals is lower than that of controls; the increases produced by intravenous glucose over fasted controls and by glucose and insulin, as compared to glucose alone, were not large; the difference between fasted controls and animals injected with glucose and insulin was quite definite.

Liver. The derived values for carcass glycogen and for total glucose have been obtained by assuming the values for gastrocnemius glycogen and for blood glucose, respectively, to be distributed through 50 per cent of body weight. The values for carbohydrate unaccounted for is then the difference between glucose retained and the sum of the increases in glycogen and glucose. These assumptions are only approximately valid but are justifiable on a comparative basis in as far as they make for more quantitative interpretation of the data obtained. It is probable that they are as valid as would be respiratory quotients obtained under these conditions.

Considering groups 2 to 6, inclusive, the striking finding is the progressive decrease in liver glycogen as the insulin dose is increased. This effect of insulin on liver glycogen has been repeatedly observed. It is commented upon in the reviews of Cori (2), Best (3) and Soskin (5). The work of Bridge (9) has recently re-emphasized it. Others who have called attention to it include Cori and Cori (10, 11), Reid (12), Bodo and Neuwirth (13), Lundsgaard (14) and Russell (15). It is again recorded in this paper because a few new experimental circumstances are attached to the primary observation. A number of objections to admitting the evidence at its face value have been or can be made and will be briefly discussed.

The phenomenon would not appear to be dependent on discharge of epinephrine because it has been observed to occur in adrenalectomized animals, not only in these experiments but also by Cori and Cori (16) and Bridge (9).

Because of the lengthy survival of the adrenalectomized animals used by Cori and Cori, the participation of the adrenal cortex was conceivable. However, the experiments of Bridge and of this paper exclude this possibility.

Hypoglycemia affecting the liver directly would appear to be ruled out by the present experiments.

On comparing groups 2 and 3, it would be possible to say that the decreased liver glycogen was accounted for by the increased muscle glycogen, by the increased oxidation which is reflected in the increase of unaccounted carbohydrate, or by a combination of the two; when, however, the figures for groups 3 to 6, inclusive, are considered, it is not easy to make out any certain tendency for a progressive increase in either as the insulin dose is increased. Furthermore, merely to account for the glycogen lost from liver as being disposed of elsewhere in no wise minimizes the fact that it *has* decreased and has done so in the face of a hyperglycemia which does not decrease with progressive increases in the amount of insulin employed. In group 7 glucose alone as in group 2 was given for 2 hours, followed, however, by an extra hour of the same glucose injection plus insulin. At the end of 2 hours it is to be supposed that liver glycogen was at the level of group 2. In the third hour despite the extra sugar injected, and which

sustained the blood glucose level, the liver glycogen dropped sharply under the influence of insulin.

As regards the possibility that insulin by reducing gluconeogenesis from protein could explain the effect, it should be pointed out that if all protein catabolism were stopped (75 mgm. urinary nitrogen per 100 grams per day, Evans (7)), only 24 of the 74 mgm. drop in liver glycogen (groups 2 and 5) could be accounted for. In addition, neither the explanation of decreased gluconeogenesis from protein, or from fat as argued for by Soskin, could account for the fact that liver glycogen has not been deposited during hyperglycemia.

In table 3 of the paper by Cori and Cori (11), for rats fed glucose and glucose + insulin, the average respiratory quotients are 0.884 and 0.903, respectively. Russell (15) records values of 0.857 and 0.887 for similar experiments. Despite the criticism of the respiratory quotients again raised by Soskin (5), it is difficult to conceive of any rapid and considerable conversion to fat occurring without a sharper rise in the respiratory quotient; it can be calculated that per 100 grams of rat, a conversion of 50 mgm. of carbohydrate to fat in 1 hour would result, other things being equal, in a rise of approximately 0.100 in the respiratory quotient. Further, to explain the fall in liver glycogen under the action of extra insulin as due to conversion to fat still leaves unexplained the failure to deposit liver glycogen from circulating glucose.

The experiments of Corey and Britton (17) on glucose-perfused liver and of Ostern, Herbert and Holmes (18) on glucose-bathed liver brei give some indication that the insulin effect here discussed may be directly upon the liver.

It may be that the findings of this paper represent the pharmacologic and not the physiologic action of insulin upon the liver; in this connection it is to be noted that the rat is not very insulin sensitive and that the smallest doses used do not greatly exceed on a weight basis the amounts occasionally used clinically; furthermore, because the experiments were short and relatively acute, the maximal effect of the insulin was probably not achieved. Should the effect be a pharmacologic one, it is nevertheless highly specific and as such is of interest.

Crystalline insulin (Lilly) was used in four of the experiments of group 5, and the results could not be differentiated from those due to ordinary insulin solution.

The experimental results of this paper do not justify an extended discussion of the perplexing problem of insulin action and the awkward facts connected with it; these have been dealt with in the competent reviews already mentioned. It is, however, appropriate to emphasize the fact that *a*, insulin lowers liver glycogen in the intact animal; *b*, progressively so with increase of insulin dosage; *c*, without mediation of the adrenal,

and *d*, despite hyperglycemia; it is also argued that *e*, suppression of gluconeogenesis or increased conversion to fat does not explain non-deposition of liver glycogen. The evidence tends to indicate that the effect is directly upon the liver.

SUMMARY

Rats were injected intravenously with glucose with or without insulin.

Cardiac glycogen was found to be increased by glucose, but not further increased by the addition of insulin.

For such glucose injected animals, the liver glycogen was found to be lowered by insulin and progressively so as the dose was increased; the effect occurs even during hyperglycemia; it is not mediated by the adrenal. Possible explanations are discussed.

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INHIBITION OF THE PYLORIC SPHINCTER REGION BY THE DIGESTION PRODUCTS OF FAT[1, 2]

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It has recently been shown by Quigley, Werle, Ligon, Read, Radzow and Meschan (1) that the presence of natural fats (cream or egg yolk) in the duodenum leads to inhibition of the entire pyloric sphincter region. Their evidence indicated that the well known delay which fats exert on gastric evacuation resulted from the cessation of antral propulsive peristalsis and occurred in spite of the relaxed pyloric sphincter and bulb. Whether this action resulted from neutral fats *per se*, from their digestion products or from both groups of compounds has not been determined. Tönnes and Nevers (2) claimed that neutral fats in the duodenum did not modify gastric evacuation and therefore only the digestion products were effective. Their report cannot be accepted for they employed no method to prevent digestion of the neutral fat they administered. Card's (3) demonstration that a variety of fat digestion products inhibited the body of the stomach is only indirectly applicable to the question of gastric evacuation.

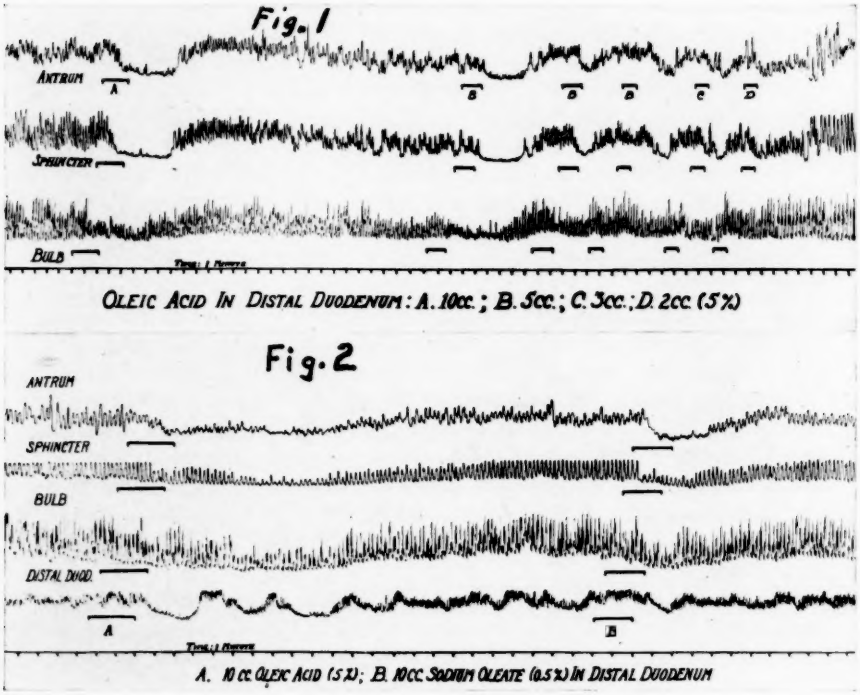
The present study constitutes the first investigation of the effects of pure fat digestion products on the motility of the sphincter region. We recorded motor activity from balloons placed in the pyloric antrum, sphincter, duodenal bulb and distal duodenum of 7 trained dogs provided with permanent cannulae opening into the stomach and duodenum (method of Meschan and Quigley (4)). The test solutions, at body temperature, were slowly introduced 18 hours post cibum into the duodenum just beyond the distal duodenal balloon.

Fatty acids. The following substances were administered in 10 cc. quantities of 5 per cent emulsions in acacia; oleic acid (40 expts.), linoleic acid (6 expts.), palmitic acid (3 expts.), ricinolic acid (8 expts.), myristic acid (5 expts.). Each of these acids inhibited the entire pyloric sphincter

[1] A preliminary report of this work was made in the Review of Gastroenterology **4:** 272, 1937.

[2] This investigation was supported in part by a research grant from the Ella Sachs Plotz Foundation.

region (fig. 1) in a manner similar to that of the natural fats. The various fatty acids always produced a decrease in frequency and magnitude of the recorded waves but displayed some quantitative variation in action. This inhibition was progressively less complete in passing from the antrum to the distal duodenum and began in 75 to 120 seconds. Maximal inhibition



Registration by the tandem balloon-water manometer technic.

Effects on the pyloric sphincter region of fat digestion products introduced into the distal duodenum.

Fig. 1. Effects of varying quantities of oleic acid.

Fig. 2. Comparison of effects from 10 cc. 5 per cent oleic acid with those from 10 cc. 0.5 per cent sodium oleate.

developed in about 3 minutes followed by complete recovery after approximately 10 minutes. A repetition of effects from a series of small doses was also demonstrated (fig. 1). The effect from 10 cc. of 5 per cent oleic, or linoleic acid was comparable to that from 4 cc. of cream (30 per cent B.F.), thus these free fatty acids exhibited about twice the inhibiting action on the sphincter region shown by the natural fat. Palmitic, ricinoleic and

myristic acids were less than one-half as effective as the other acids in this series (i.e., they were about as active as the natural fats). The inhibition from these three fatty acids, especially ricinoleic acid, frequently was less complete than from cream, and of approximately half the duration. The latent period was shortest with myristic acid (1 min.) and longest with ricinoleic acid (8 min.). The injection of 20 cc. quantities of the fatty acid emulsions increased only the duration of the inhibition and this increase was moderate.

Soaps. Sodium oleate, 10 cc. 0.5 per cent (15 expts.), sodium myristate 10 cc. 0.5 per cent (5 expts.), and sodium palmitate 10 cc. 2 per cent (4 expts.) produced marked inhibition of the sphincter region (fig. 2), but the depression was less complete and of only half the duration of 10 cc. of cream. Sodium ricinoleate 10 cc. 5.0 per cent (9 expts.) produced only moderate inhibition. The effects from sodium butyrate 10 cc. 5.0 per cent (9 expts.) were variable, with some tendency toward augmentation of bulbar activity. The early effect of sodium myristate on the bulb was augmentory, but this was usually followed by an inhibition.

Placed in order of their decreasing effectiveness, the compounds in our series were oleate, myristate, palmitate and ricinoleate. This order applied to the free acid or to the sodium soap. The soaps appeared to be approximately ten times as effective as the free fatty acids, but the doses employed did not give sufficiently comparable results to permit a strict comparison.

Emery and Edwards (5) found the irritant action of sodium soaps on the human skin decreased in the following order: myristate, oleate, ricinoleate, palmitate. Thus the sphincter-inhibiting action of soaps probably does not depend on its irritating action. Oleic acid and ricinoleic acid have the same number of carbon atoms and both are unsaturated compounds, but oleic acid is a much more effective depressant of the sphincter region than is ricinoleic acid. Our results afford no confirmation of the report of Roberts (6) that the delay in evacuation is related to the degree of unsaturation of the fat employed.

Glycerol and mineral oil. The administration of 10 cc. quantities of 0.5 to 2.5 per cent glycerol solution (18 expts.) produced variable effects on the sphincter region. All the effects were slight and favored the conclusion that such quantities of glycerol were without specific effect. Similar quantities of 3 to 5 per cent glycerol produced nausea and vomiting.

Heavy mineral oil (7 expts.) was used undiluted in 10 cc. quantities. No alteration in the motility of the sphincter region resulted, thus indicating that the inhibitory actions of fats were not dependent on the physical properties of oils. This was in accord with the observation of Edelman (3) that vaseline in the intestine failed to inhibit gastric evacuation.

Discussion. The inhibition of the pyloric sphincter region following the intraduodenal administration of soaps or fatty acids is qualitatively similar to that produced by natural fats. The action progressively diminishes from the antrum to the distal duodenum. The retardation of gastric evacuation produced by the entrance into the duodenum of fats or their digestion products apparently results from a decreased antral peristalsis and occurs in spite of a relaxed sphincter.

In producing inhibition of the sphincter region, the fatty acids were 1 to 2.5 times as effective as the natural fats. The soaps varied greatly from sodium butyrate which was less effective than cream to sodium oleate and sodium myristate which were approximately 30 times more effective.

Our experiments bear on the question whether fats *per se* inhibit the sphincter region or only become active after digestion, but they do not supply a positive answer. Only a few of the digestion products of fats used in our experiments were more effective than the natural fats, the others were either less active or equal in activity to the fats. Moreover, the latent period of 75 to 100 seconds was the same with either fats, fatty acids or soaps. If only fat split products were active, neutral fat could only become effective after it had undergone digestion and a significant concentration of the products had come in contact with the mucosa and initiated the influence on the sphincter region. We might assume that under favorable conditions this series of events could occur within the latent period noted for fats. However, the latent period was within these limits even under conditions which should retard the process: *a*, when 30 cc. of cream was employed, the enzyme and digestion products were markedly diluted and mechanically kept away from the mucosa, or *b*, when the pancreatic juice and bile were sucked out of the proximal duodenum and blocked by a balloon from entering the distal portion of the duodenum into which the cream was introduced. Thus our observations favor the conclusion that natural fats initiate the sphincter region inhibition and the action is continued by the fatty acids and soaps formed during digestion, but the glycerine is inactive.

SUMMARY

Studies made with the tandem balloon method demonstrated that the introduction of fatty acids or soaps into the proximal intestine of fasting dogs inhibited the motility of the pyloric antrum, sphincter and duodenal bulb in a manner qualitatively similar to that produced by natural fats. Usually the order of decreasing activity was soaps, fatty acids, natural fats. However, the evidence indicated that if natural fats were administered, they initiated the inhibition and the digestion products continued the influence.

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ROENTGENOGRAPHIC OBSERVATIONS SUGGESTING DIFFERENCE BETWEEN TOTAL AND CIRCULATING BLOOD VOLUME

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Reduction in measured blood volume after various constricting drugs has been reported by various observers, including Freeman (1) after adrenalin, Griffith, Comroe and Zinn (2) after ergotamine tartrate, and Griffith, Corbit, Rutherford and Lindauer (3) after pressor doses of pitressin. In the latter study in a series of 10 rats blood volume, as measured by the dye method of Griffith and Campbell (4), was found to be so greatly reduced that the results were scarcely credible.

If the peripheral capillaries of the rat's foot are observed in such an experimentally treated animal it is seen that, as a rule, blood cells remain in the capillaries but the flow stops. As this observation could apply only to a limited number of vessels of a peculiar type, the skin capillaries, it was decided to extend it to larger vessels using intravenously injected thorotrast and x-ray. Moreover, we planned to follow blood volume changes by other methods,—by hematocrit, hemoglobin and plasma protein determinations.

METHOD. A. *Observations of the general vascular tree of the rat by x-ray.* Large albino rats were used, usually weighing 300 grams or more. Under nembutal anesthesia one of the larger veins was exposed, usually one of the femoral veins. A small hypodermic needle was inserted and an amount of blood was withdrawn which was intended to equal the amount of thorotrast which was to be given, usually 3 cc. It was not always possible to withdraw quite this much blood. However, in preliminary experiments when blood was not withdrawn it appeared that there was little difference in the vascular shadows, although the animal in general was not in as good condition. Immediately after the injection of thorotrast, fluoroscopic observation was begun and, at intervals, roentgenograms were made.

Two procedures were followed: 1. *The thorotrast-pitressin procedure.* The thorotrast was given first and fluoroscopic observations begun. Then

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5 pressor units of pitressin were given intraperitoneally and the fluoroscopic observation continued for 30 minutes. Five animals were used.

2. *The pitressin-thorotrast procedure.* The same dose of pitressin was given, followed in 30 minutes by the thorotrast. Then fluoroscopic observation was continued for 20 to 30 minutes. Several animals died shortly after the intravenous injection of thorotrast, but five survived and appeared to be in satisfactory condition.

B. In 5 normal albino rats hemoglobin, hematocrit, and plasma protein measurements were made immediately before and 30 minutes after the intraperitoneal injection of 5 pressor units of pitressin. The technique for these measurements in rats has been previously reported (5,6). Dilution or concentration of blood volume is expressed in terms of per cent of the original measurement. The results obtained were compared with those previously reported (3) in which blood volume was measured several days before and again 30 minutes after giving the pitressin. At the time of each measurement vital red was injected into the jugular vein and its concentration measured in heart blood obtained 4½ minutes later.

RESULTS. A. *Thorotrast-pitressin procedure.* The artery and corresponding vein show as a single vascular shadow. The general vascular bed was well outlined by the thorotrast, including vessels to the extremities. After the injection of pitressin there was an abrupt decrease in the size and increase in the rate of the heart, occurring within a few seconds, with little other appreciable effect.

Pitressin-thorotrast procedure. The heart was somewhat small when first observed. The central vessels of the body, including those of the thorax, abdomen and kidney, were shown as well as or even better than in the previous procedure, but the vessels to the limbs were seen faintly if at all. Ten minutes or more after the injection of thorotrast the vessels of the limbs showed more distinctly but not to the extent seen in animals in the thorotrast-pitressin procedure.

B. Figure 1 shows the result of estimating change in blood volume by various methods. The change in hematocrit reading 30 minutes after pitressin indicated a change in blood volume ranging from an 11 per cent concentration to a 42 per cent dilution, but averaging a 14 per cent dilution or increase in blood volume. The change in hemoglobin indicated an average change in blood volume of only 2 per cent dilution, but the range was from 11 per cent concentration to 28 per cent dilution. Based upon change in plasma protein the blood volume appeared to have increased by 1 per cent, but the range was from 33 per cent concentration to 21 per cent dilution. It should be noted that in all instances the blood for the second sample was secured from the heart, as after pitressin blood cannot be obtained by cutting a tail vein. The preliminary sample was obtained in

2 instances from the heart and in 3 from a tail vein. In normal animals samples obtained by either route are essentially identical.

These figures must be compared with those previously obtained (3) using vital red. This method indicated an average reduction of blood volume by 38 per cent, ranging from 10 to 60 per cent.

Discussion. It is known that blood cells may remain for considerable periods within the minute vessels of the spleen, liver, and bone marrow without entering into the general circulation. However, they may enter into the general circulation, either gradually or suddenly, when physiologic need arises. Some plasma must also be retained with the cells, but

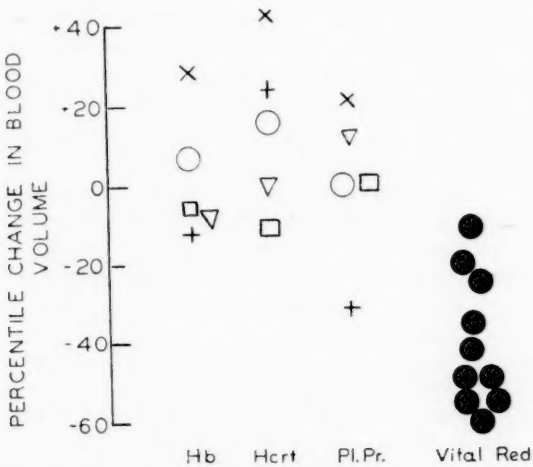

Fig. 1. Percentile change in blood volume after pitressin estimated in ten rats (●) by repetition of vital red blood volume determination and in five other rats, each designated by a particular symbol, by each of three methods, change of hemoglobin, of hematocrit, and of plasma protein concentration.

it is uncertain whether the amount is as great as though the cells were actually circulating. Blood retained in such vascular reservoirs would not be included in measurements of blood volume made by a dye method. If, for any reason, more of the circulating blood was withdrawn into such reservoirs, provided both cells and plasma were withdrawn proportionately, blood volume as measured by a dye method would be greatly decreased but the blood volume as estimated from hematocrit, hemoglobin or plasma protein measurements would be unchanged. It is conceivable that, under conditions of intense vasoconstriction, circulation to certain parts or organs may be arrested to the extent that they, too, serve as reservoirs for non-circulating blood. Even if the blood in such parts or organs does

circulate, but very sluggishly, the mixing time for dye used in blood volume measurements may be so greatly increased over the normal that, if the interval for the normal be used, all figures obtained will be too low.

To recapitulate, under conditions of vasoconstriction, fluid may be lost from the blood, resulting in some degree of hemoconcentration and lowered blood volume. Such a lowering of blood volume could be demonstrated by serial determinations of hemoglobin, hematocrit values, plasma protein, or by a dye method for measuring blood volume. On the other hand, if vasoconstriction results in trapping of blood in areas without active circulation, changes in blood volume will be estimated as slight if based on hemoglobin, hematocrit, or plasma protein measurements, but great if based upon a dye method, since this measures circulating blood volume rather than total blood volume. This latter phenomenon appears to have occurred in the experiments here recorded.

SUMMARY

After a strong vasoconstricting agent, pitressin, blood volume measured by a dye method is greatly reduced. We suggest that a considerable amount of blood may be trapped in areas of the peripheral circulation so that actively circulating blood volume may be much less than total blood volume. The blood still circulating shows relatively slight changes in hematocrit red cell volume or in plasma protein concentration. The presence of blood vessels in the extremities containing blood but without active circulation is shown by the following: 1. By microscopy, the skin capillaries contain red cells, but there is no flow. 2. Thorotrast introduced into the general circulation before giving the pitressin remains in and outlines the vessels of the extremities. 3. Blood cannot be secured by cutting a tail vein. 4. Thorotrast introduced into the general circulation after the injection of pitressin either does not enter into and outline the vessels of the extremities or does so tardily and to a lesser extent.

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